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GRANT NUMBER: DAMD17-95-1-5040

TITLE: Overview of Developments and Current Applications of In Vitro Toxicology (In Vitro Biology Workshop 20-24 May 1995, Denver, CO)

PRINCIPAL INVESTIGATOR: John W. Harbell, Ph.D.

CONTRACTING ORGANIZATION: Society for In Vitro Biology
Columbia, MD 21045

REPORT DATE: October 1995

TYPE OF REPORT: Final Proceedings

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

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12a. DISTRIBUTION / AVAILABILITY STATEMENT
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13. ABSTRACT *(Maximum 200 words)*

Enclosed is the program issue of the Congress on In Vitro Biology, 1995 Meeting of the Society for In Vitro Biology. The theme of the Congress was Interplay of Cells with Their Environment. An exciting plenary session started the Congress: the speakers included Drs. Michael Strand, Donald E. Ingber, Ilya Raskin and E. Elmore. Topics which gave an overview of developments and current applications of In Vitro Toxicology are as follows: Novel Insights Into Chemical Neurotoxicity; PCR Methods in Toxicology; Cellular and Molecular Toxicology; Mediators of Inflammation and Immunotoxicology; In Vitro-In Vivo Correlations in Toxicology and Transportand Biotechnology: Models and Methods.

14. SUBJECT TERMS
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Vertebrate Cells
Invertebrate Cells
Plant Cells

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151

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17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
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John Harbell 10-16-95

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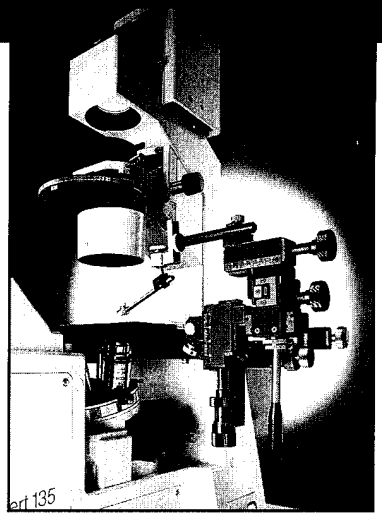

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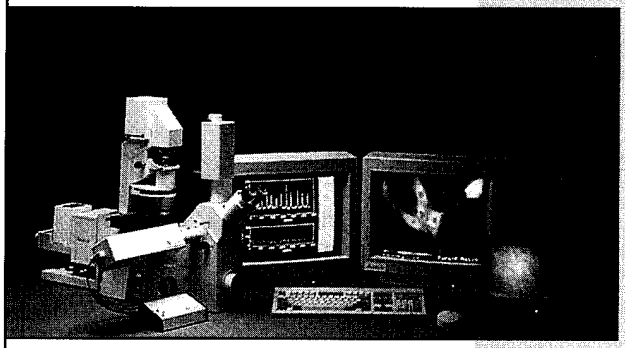

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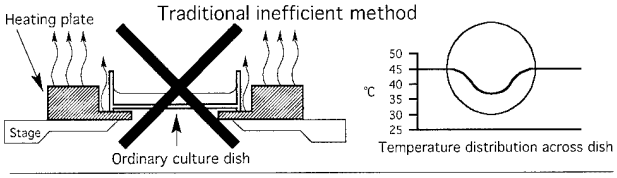

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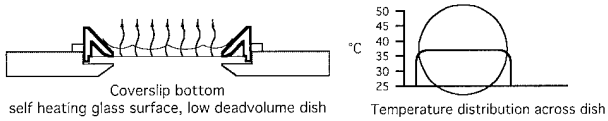

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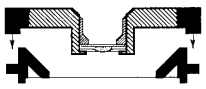
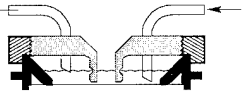
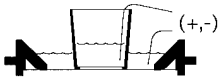
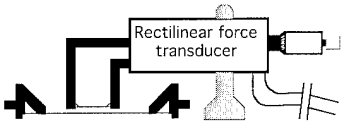

Brain slice micro-observation chamber

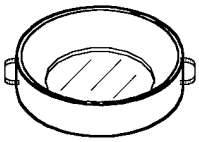

Tissue slice chamber

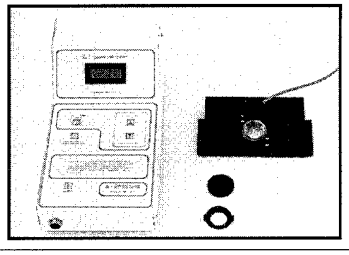


Artificial membrane insert micro-observation chamber

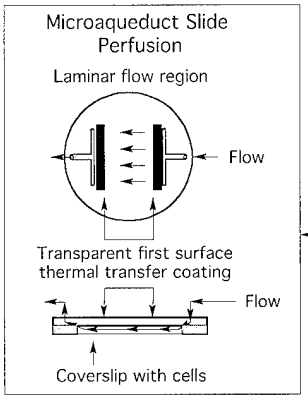


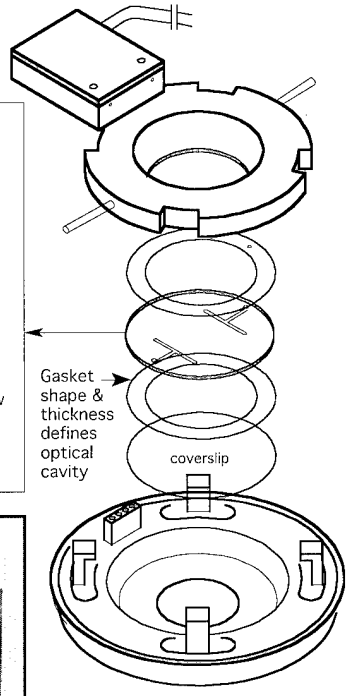
Isometric muscle slice force transduction


Think of it as a precision miniature glass bottomed hot-tub for your cells!



Closed System FCS2





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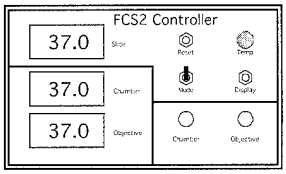

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IN VITRO
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VOLUME 31 NUMBER 3 PART II
ISSN 1071-2690
MARCH 1995

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IN VITRO ANIMAL
CELLULAR & DEVELOPMENTAL BIOLOGY

Journal of the
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In Vitro Cellular & Developmental Biology—Plant (ISSN 1054-5476) is published quarterly (one volume, four issues per year) by the Society for In Vitro Biology (SIVB), 8815 Centre Park Drive, Suite 210, Columbia, MD 21045. *In Vitro Cellular & Developmental Biology—Plant* is devoted to publishing original papers in plant cellular and developmental biology with emphasis on the developmental, molecular and cellular biology of cells, tissues, and organs. Manuscripts reporting the use of plant tissue culture techniques in plant breeding, horticulture, plant physiology, molecular biology, and other plant disciplines are also accepted. Non-member subscription rate is: $105.00 per volume ($135.00 outside North America via surface mail), payable in advance in U.S.A. funds drawn on a U.S.A. bank. If not a U.S.A. bank, add $25.00 bank clearance fee. Claims of nonreceipt should be made within six months of publication. Issues claimed after six months may be purchased as back issues at $15.00 per issue. SIVB Plant Cellular and Developmental Biology Division members receive *In Vitro Cellular & Developmental Biology—Plant* ($25.00) as part of annual dues. Correspondence relating to subscriptions, nonreceipt of journals, back issues, advertising, and late or lost proofs should be directed to Marietta Ellis, Business Manager, Society for In Vitro Biology, 8815 Centre Park Drive, Suite 210, Columbia, MD 21045 USA (410)992-0946. Second-class postage paid at Columbia, MD, and additional mailing offices.

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1995 Congress on In Vitro Biology
Schedule of Functions

Time	Type of Function	Room
Friday, May 19		
8:00 am	SIVB Executive Board Meeting	TBA
Saturday, May 20		
1:00 pm	Program Committee Meeting	Denver Room
2:00 pm	SIVB Council Meeting	Silver Room
4:00 pm	SIVB History Society Meeting	Grand Ballroom D&E
4:30 pm	Cellular Toxicology Committee Meeting/Reception	Century-Spruce Rooms
7:00 pm	Congress Opening Reception	Grand Ballroom A-C
Sunday, May 21		
10:00 am	**Exhibits and Posters** (10:00 am-6:00 pm)	Exhibit Hall
1:30 pm	Cell Culture Standardization Committee Meeting	Capitol Room
1:30 pm	Publications/Editorial Committee Meeting	Terrace Room
5:30 pm	Student Social	Terrace Room
Monday, May 22		
7:00 am	Plant Division Program Breakfast Meeting	Denver Room
10:00 am	**Exhibits and Posters** (10:00 am-6:00 pm)	Exhibit Hall
12:30 pm	Lunch Break Co-sponsored by Exhibitors and SIVB	Exhibit Hall
1:30 pm	Constitution and Bylaws Committee Meeting	Terrace Room
4:45 pm	Vertebrate Division Committee Meeting	Terrace Room
5:00 pm	Happy Hour	Exhibit Hall
5:00 pm	Strategic Long Range Planning Committee	Capitol Room
5:00 pm	Plant Division Business Meeting	Denver Room
6:00 pm	SIVB Plant Division Social	Pool Side
7:00 pm	Special Education Workshop	Silver Room
Tuesday, May 23		
9:00 am	Development Committee Meeting	Capitol Room
10:00 am	**Exhibits and Posters** (10:00 am-5:00 pm)	Exhibit Hall
12:00 pm	Invertebrate Division Luncheon Meeting	Capitol Room
1:30 pm	Membership Committee Meeting	Terrace Room
1:30 pm	Laboratory Materials & Biosafety Meeting	Savoy Room
5:00 pm	SIVB Business Meeting	Silver Room
7:00 pm	Congress Awards Banquet	Grand Ballroom
Wednesday, May 24		
10:30 am	Program Committee Meeting	Century Room

NOTE: Additions and changes to functions will be posted on a bulletin board located in the registration area. Please check the bulletin board daily.

PLAN TO VISIT THE EXHIBITS

Sunday — 10:00 am-6:00 pm
Monday — 10:00 am-6:00 pm
Tuesday — 10:00 am-5:00 pm

Morning coffee breaks Sunday, Monday, Tuesday at 10:00 am.
Lunch Break co-sponsored by Exhibitors & SIVB Monday at 12:30.
Happy Hour Monday at 5:00 pm

Congress on In Vitro Biology

1995 Meeting of the Society for In Vitro Biology

May 20–24 • Denver, CO

Interplay of Cells With Their Environment

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Washington State University

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FRIDAY, MAY 19

8:00 am-5:00 pm	**SIVB EXECUTIVE BOARD**	**TBA**

SATURDAY, MAY 20

9 am-8 pm	**REGISTRATION**	**South Convention Lobby**
1:00-2:00 pm	**PROGRAM COMMITTEE MEETING**	**Denver Room**
2:00-3:30 pm	**SIVB COUNCIL MEETING**	**Silver Room**
3:00-6:00 pm	**POSTER SET UP**	**Exhibit Hall**
4:00-6:00 pm	**HISTORY SOCIETY** *Conveners:* Len Schiff and James Henderson	**Grand Ballroom D&E**

History: Development of Methods for Studying Tissue Organization in Culture
J. LEIGHTON

History of the CHO Cell
R.G. HAM

Development of Mosquito Cell Culture
J. MITSUHASI

SATURDAY, MAY 20

4:30-6:00 pm	CELLULAR TOXICOLOGY COMMITTEE BUSINESS MEETING/RECEPTION	Century-Spruce Rooms
7:00-9:00 pm	OPENING RECEPTION	Grand Ballroom A-C

Numbers preceeding names refer to abstracts.
Capitalization identifies speaker.

46th Annual Meeting of the Society for In Vitro Biology

Key Letters Preceeding Session Title

I = Invertebrate Cells
P = Plant Cells
PS = Plenary Session
T = Cellular Toxicology
V = Vertebrate Cells
W = Workshop
JS = Joint Symposium

SUNDAY, MAY 21

7:00 am-7:30 pm — **REGISTRATION** — **South Convention Lobby**

INTERPLAY OF CELLS WITH THEIR ENVIRONMENT

Convener: Howard L. Hosick, Washington State University

Co-sponsored by Collaborative Biomedical Products/Becton Dickinson Labware

8:00-10:00 am — **PLENARY SESSION** — **Grand Ballroom**

(See abstracts on page 1A)

8:00	PS-1	The Cellular Immune Response of Insects: *In Vitro* Approaches to the Study of Antiviral and Antiparasitic Defense Mechanisms MICHAEL STRAND, University of Wisconsin
8:30	PS-2	Control of Cell Growth and Differentiation By Extracellular Matrix DONALD E. INGBER, Children's Hospital/Harvard Medical School
9:00	PS-3	Do Plants Take Aspirin? ILYA RASKIN, Rutgers University/Cook College
9:30	PS-4	Developing Valid *In Vitro* Alternatives for Toxicology and Pharmacology E. ELMORE

10:00-10:30 am — **COFFEE BREAK** — **Exhibit Hall**

10:00 am-6:00 pm — **EXHIBITS AND POSTERS** — **Exhibit Hall**

Even Numbered Poster Authors Will
Be Present 12:30-1:30 pm
Odd Numbered Poster Authors Will
Be Present 4:30-5:30 pm
(See list of Posters on pages xxv-A–xxxiv-A

EXTRACELLULAR MATRIX AND CELL BEHAVIOR *(V)*

Convener: Brigitta Tadmor, Collaborative Biomedical Products/Becton Dickinson Labware

Keynote Speaker: Stephen R. Farmer, Boston University School of Medicine

Sponsored by Collaborative Biomedical Products/Becton Dickinson Labware

10:30 am-12:30 pm — **SYMPOSIUM** — **Majestic Ballroom**

(See abstracts on page 6A)

10:30		Introduction (B. Tadmor)
10:35	V-1	Cell-Extracellular Matrix Interactions and Control of Tissue-Specific Transcription Factors S.R. FARMER
11:05	V-2	To Be Announced H. REDDI
11:35	V-3	Dynamic Reciprocity Revisited: A Continuous, Bidirectional Flow of Information Between Cells and the Extracellular Matrix Regulates Mammary Epithelial Cell Function C.D. ROSKELLEY and M.J. Bissell

APPLICATIONS OF MODELS OF HEPATOTOXICITY *(T)*

Convener: Jack Lipman, Hoffmann-La Roche
Keynote Speaker: D. Acosta, Jr., University of Texas
Sponsored by Industrial In Vitro Toxicology Group

10:30 am-12:30 pm **SYMPOSIUM** **Columbine Room**

(See abstracts on page 12A)

10:30		Introduction (J. Lipman)
10:35	T-1	An *In Vitro* Approach to the Study of Hepatotoxic Agents with a Primary Culture System of Rat Liver Cells D. ACOSTA, JR.
11:05	T-2	To Be Announced C. RUEGG
11:35	T-3	Cultured Human Hepatocytes *In Vitro* Models for Examining Drug Toxicity and Metabolism R. ULRICH

INTERPLAY OF PLANT CELLS WITH THEIR ENVIRONMENT: THE ROLE OF STRESS-INDUCED PROTEINS *(P)*

Conveners: Ron Newton, Texas A&M University; John Finer, Ohio State University

The growth and development of plants are constantly under the influence of the environment. Much research in the last several years has concluded that the response to the environment involves the regulated expression of a number of genes, producing a variety of proteins. Hormones play a pivotal role in some responses, and it is clear that signal transduction pathways which control the expression of genes overlap in response to a variety of different environmental cues. Promoter analysis indicates sequence-specific regions that are responsive to various external signals. In addition, current research is focused on the isolation and characterization of trans-acting factors that participate in environmentally-induced transcriptional activation, as well as the role of post-transcriptional and post-translational regulatory mechanisms. This session will focus on recent developments in the understanding of signal transduction, and the transcriptional and translational responses of the genetic machinery of cells to environmental stresses.

10:30 am-12:30 pm **SYMPOSIUM** **Grand Ballroom A-C**

(See abstracts on pages 17A–18A)

10:30	P-1	Osmotically-induced Plant Defense Genes: Structure and Function R.A. BRESSAN
10:55	P-2	Desiccation-induced Protein Synthesis: A Role in Cellular Repair? M.J. OLIVER
11:20	P-3	Regulation of Environmental Stress- and Abscisic Acid-induced Genes T.-H.D. HO
11:45	P-4	Role of the Tobacco Anionic Peroxidase in Growth and Development L.M. LAGRIMINI
12:10	P-5	Molecular Aspects of Crassulacean Acid Metabolism: An Adaptation to Environmental Stress J.C. CUSHMAN, H.J. Schaeffer, N.R. Forsthoefel

THE INTERACTION OF INVERTEBRATE CELLS WITH TOXINS AND THEIR ENVIRONMENT *(I)*

Convener: James Vaughn, USDA/ARS

Meaningful *in vitro* studies on the effects of toxins and other environmental stress factors depend on the ability to culture cells from a variety of tissues and animals. Until recently, the limited sources of cultured invertebrate cells has restricted toxicology studies. The papers in this symposium not only report interesting results in *in vitro* studies, but show the considerable progress in the culture of the range of tissues from invertebrates that are needed if the *in vitro* studies are to relate to the whole animal.

10:30 am-12:30 pm **SYMPOSIUM** **Vail Room**

(See abstracts on pages 21A–22A)

10:30		Introduction (J. Vaughn)
10:35	I-1	Factors Influencing Cellular Interactions in the Developing Antennal Lobe of the Moth, *Manduca Sexta.* L.A. OLAND
11:05	I-2	Effect of the CryIA Group of *Bacillus thuringiensis* Toxins on Midgut Epithelial Cells and Insect Cell Lines from Lepidopteran Larvae D. BAINES, J.-L. Schwartz, S. MacIntosh, O. Thastrup
11:35	I-3	Mechanisms of Radiation Resistance in Lepidopteran Insect Cells T.M. KOVAL
12:05	I-4	A Rotenone-Resistant Cell Line and Its Specific Characteristics J. MITSUHASHI and Y. Yanagimoto
12:35	I-5	Environmental Effects on Oyster Hemocytes in Primary Culture W.S. FISHER
1:05	I-6	Use of Sponge Cell Cultures as Environmental Indicators of Pollution S.A. POMPONI, R. Willoughby, M.E. Kaighn, H. Zhong

12:30-1:30 pm **POSTER PRESENTATION** **Exhibit Hall**

Even Numbered Poster Authors Present
(See list of Posters on pages xxv-A–xxxiv-A)

INTERCELLULAR EVENTS IN SIGNAL TRANSDUCTION AND GENE CONTROL *(V)*

Convener: Sandra L. Schneider, University of Texas Health Science Center
Keynote Speaker: Dean Edwards, University of Colorado Health Science Center

Regulation of cellular survival and differentiation of discrete and overlapping cell populations involve the mechanisms of signal transduction. Initiation of signaling events that lead to cellular differentiation are either through receptor-induced tyrosine phosphorylation or alterations of specific protein conformation. This Symposium will present a review of the steroid hormones and associated intracellular receptors that represent a major signal transduction pathway by which extracellular molecules regulate gene expression. The model systems to be discussed include the human progesterone nuclear reactor (PR) in breast cancer cells and tissue specificity of this steroid response; specific activated kinases required for mitogenic signaling of IL-3 receptor in hematopoietic cells; and the identification of oncogene signaling cell specific factors that control selective gene expression.

2:30-4:30 pm **SYMPOSIUM** **Majestic Ballroom**

(See abstracts on pages 31A–39A)

2:30 Introduction (S.L. Schneider)

2:35	V-4	Steroid Receptor Signaling Mechanisms D.P. EDWARDS
3:05	V-5	Coupling of Multiple Signal Transduction Pathways with Steroid Hormone Response Mechanisms: Implications for the Nuclear Receptor Family and Tissue Specificity of Hormone Response S.K. NORDEEN
3:35	V-6	Signal Transduction by the Receptor for Interleukin-3 S.M. ANDERSON
4:05	V-7	Oncogene Signaling: Identification of Cell-Specific Factors Controlling Selective Gene Expression A. GUTIERREZ-HARTMANN, A.P. Bradford, K.E. Conrad, B. Wasylyk

ORGAN-SPECIFIC CELLULAR MODELS *(V)*

Moderator: Steve Benson, California State University, Hayward

2:30-4:30 pm **CONTRIBUTED PAPERS** **Vail Room**

2:30	V-1001	Extracellular Matrix Modulation of the Cellular and Molecular Aspects of Myogenesis G. Bahador, A. Davalos, S. BENSON
2:50	V-1002	Expression of Type VI Collagen During Glioblastoma Cell Invasion in Brain Tissue Cultures J. Han and J.C. DANIEL
3:10	V-1003	Application of a Composite Skin Graft to Nude Mice: Dynamic Interaction Between Cells of the Epidermis and the Dermal Graft E.S. GRIFFEY and S.A. Livesey
3:30	V-1004	A Novel Three-Dimensional Liver Culture System With Applications to Transplantation and Extracorporeal Liver Assistance B. SIBANDA, J. Gee, J. San Román, V. Kamali, B.A. Naughton
3:50	V-1005	A Method for the Primary Culture of Epithelial and Stromal Cells from Normal Rat Dorsal Prostate and from Rat Prostate Carcinomas M.S. CONDON and M.C. Bosland
4:10	V-1006	Nitric Oxide Production in Lymphatic Endothelial Cells *In Vitro* L.V. LEAK, E.E. Just

IN VITRO-IN VIVO CORRELATIONS IN TOXICOLOGY AND TRANSPORT *(T)*

Conveners: Ken Audus, The University of Kansas; Hank Lane, Corning Costar Corp.

Sponsored by Corning CoStar Corporation

The topics of discussion in this symposium are all centered on the development of *in vitro-in vivo* correlations in toxicology and transport with an emphasis on the appropriate roles of *in vitro* models in the study of biological systems. Following a general discussion outlining the problems in developing *in vitro-in vivo* correlations in biological systems, subsequent presentations will be introduced which focus on the status of specific ongoing studies in academic and industrial laboratories. These discussions will highlight work on *in vitro* models developed for the blood-brain barrier, gastrointestinal epithelium, and the liver. The contents of the presentations under these subjects will include applications of the *in vitro* models to the study of permeability, transport, toxicological, and metabolic features in the context of corresponding *in vivo* observations.

2:30-4:30 pm **SYMPOSIUM** **Columbine Room**

(See abstracts on pages 12A–13A)

2:30 Introduction (K. Audus)

2:35	T-4	Cell and Tissue Systems *In Vitro:* The Next Best Thing to Being *In Vivo*? K.L. AUDUS
3:05	T-5	P450 Induction and Bile-Acid Transport in Cultures of Sandwiched Rat Hepatocytes E.L. LECLUYSE, P.L. Bullock, A. Parkinson
3:35	T-6	*In Vitro-In Vivo* Blood-Brain Barrier Permeability Correlations of Receptor Selective, Opiod Peptides T.P. DAVIS, V.J. Hruby, T.J. Abbruscato
4:05	T-7	Drug Transport Studies in the Intestinal Epithelial Cell Line CaCo-2: Correlation with Absorption *In Vivo* C.A. BAILEY

TRANSFORMATION AND TRANSGENIC PLANTS *(P)*

Moderator: Dennis Gray, University of Florida

2:30-4:30 pm **CONTRIBUTED PAPERS** **Grand Ballroom A-C**

(See abstracts on pages 49A–50A)

2:30	P-1001	Germline Transformation of Maize Following Particle Bombardment of Meristems M. ROSS, K. Lowe, B. Bowen, D. Tomes, G. Hoerster, L. Church, L. Tagliani, D. Bond, D. Pierce, W. Gordon-Kamm
2:45	P-1002	Microprojectile DNA Delivery to Orchardgrass Leaf Cells P.D. DENCHEV, D. Songstad, B.V. Conger
3:00	P-1003	Transformed Progeny via Particle Bombardment of Embryogenic *Cucumis melo* 'Eden Gem' Cotyledons D.J. GRAY, E. Hiebert, C.M. Lin, K.T. Kelley, M.E. Compton, V.P. Gaba
3:15	P-1004	Stable Genetic Transformation of Grapevine: Efficiency of Insertion of a Marker Gene in a R_o Population L. MARTINELLI and G. Mandolino
3:30	P-1005	Production of Fertile Transgenic Peanut Plants Using *Agrobacterium tumefaciens*, and the Expression and Inheritance of Foreign Genes in Transgenic Peanut Plants M. CHENG, A. Xing, Z. Li, R.L. Jarret, J.W. Demski
3:45	P-1006	Genetic Engineering of Peanut (*Arachis hypogaea* L.) K.K. SHARMA and J.P. Moss
4:00	P-1007	Accumulation of the 10 kD and 15 kD Zeins in Transgenic Plants S. BAGGA, F. Rodriguez, N. Klypina, J.D. Kemp, C. Sengupta-Gopalan
4:15	P-1008	Cloning and Expression of Rice Tungro Spherical Virus Proteins and Introducing Genes into Rice Tissue Y. YAN, T.M. Burns, J.W. Davies, R. Hull

MICROCULTURE RESEARCH STRATEGIES *(P)*

Moderator: Valerie Pence, Cincinnati Zoo & Botanical Garden

2:30-4:30 pm **CONTRIBUTED PAPERS** **Grand Ballroom D&E**

(See abstracts on pages 51A–52A)

2:30	P-1009	*In Vitro* Plant Regeneration and Advanced Micropropagation Methods for Pineapple E. FIROOZABADY, J. Nicholas, N. Gutterson
2:45	P-1010	Laser-Assisted *In Vitro* Biology J. CONIA and L. Keenan
3:00	P-1011	*In Vitro* Collection (IVC)—Effects of Technique and Media on Sterility and Growth of Cultures V.C. PENCE and B.L. Plair
3:15	P-1012	Regeneration of Whole Plants of *Arachis hypogea* L. from the Shoot Apex M.E. HEATLEY and R.H. Smith

3:30	P-1013	Micropropagation of Birch (*Betula pendula* Roth.) cv. Purple Rain from Shoot Tip and Bud Explants K. PRUSKI and M. Younus
3:45	P-1014	Genetic Stability of Micropropagated Strawberries M.B. KUMAR
4:00	P-1015	Antiauxin Effects on Morphogenesis in Cultures of the Endangered Cactus *Aztekium ritteri* I. REYES, J.F. Hubstenberger, G.C. Phillips
4:15	P-1016	Effect of Kinetin and BA on the *In Vitro* Culture of Potato Nodal Explants M.A. BUSTAMANTE and S. Pérez

5:30-6:30 pm **POSTER PRESENTATION** **Exhibit Hall**

Odd Numbered Poster Authors Present
(See list of Posters on pages xxv-A–xxxiv-A)

5:30-6:30 pm **STUDENT SOCIAL** **Terrace Room**
Students Are Encouraged to Attend

SUCCESSFUL STRATEGIES FOR GOOD LABORATORY (GLP) AND GOOD MANUFACTURING (GMP) PRACTICE *(T)*

Conveners: Sandra L. Schneider, University of Texas Health Science Center; Jess Stengel, Clonetics Corp.

Co-sponsored by the Baker Company, Eagleson Institute, and Clonetics Corporation

Hosted by the Laboratory Materials and Biosafety Committee, Toxicology Committee, Cell Standardization Committee, and Vertebrate Division Committee

The primary objective of this workshop is to provide instruction in the principles, methods, and application of Good Laboratory Practice (GLP) and Current Good Manufacturing Practice (cGMP). The workshop will emphasize both basic and advanced concepts of both GLP, cGMP, and ISO 9000 with regard to internationally recognized standards. Requirements and application of GLP regulations as imposed by the Food and Drug Administration (FDA), Environmental Protection Agency (EPA), and Organization for Economic Cooperation Development (OECD) will be reviewed. The application of cGMP in the pharmaceutical and biotechnology industries will include issues related to methodology, manufacturing practice and validation, quality control (QC) and quality assurance (QA), and training of personnel. Participants should: improve their knowledge and understanding of the requirements for GLP; have gained an understanding of cGMP regulations; and have a working knowledge of the similarities and differences between cGMP and ISO 9000. Discussion section will provide an opportunity for participants to seek advice on establishing and/or enhancing specific Quality Assurance Programs.

7:00-9:00 pm **WORKSHOP** **Columbine Room**

7:00		Introduction to Good Laboratory Practice: "A New Song – An Old Tune" (S.L. Schneider)
7:05	W-1	Strategies for Successful Compliance with the Good Laboratory Practice (GLP) Regulations D.S. GOLDMAN
7:35		Introduction to Good Manufacturing Practice: "The Leadership Role of the Biotechnology Corporation" (J. Stengel)
7:40	W-2	Application of cGMP Disciplines in Non-regulated Biotech Environments J.L. WEST

8:10	W-3	ISO-9001: The International Challenge G. KNEBEL
8:30		Open Discussion

MICROPROPAGATION: INTERPLAY WITH THE MICROBIAL ENVIRONMENT *(P)*

Conveners: Barbara Reed, USDA/ARS; Paul Read, University of Nebraska

Co-sponsored by Sigma Chemical Company

The presence of bacterial and fungal contaminants in plant tissue culture continues to cause problems for both commercial and research laboratories. Improved methods of indexing, identification, and treatment are being developed and are now available for use. This workshop will present information on the options available for indexing plant tissue cultures, techniques for preliminary identification and determination of proper treatment of infected cultures, and antibiotics available for treating plant tissue cultures. Discussion periods will follow each presentation for contributions from the audience.

7:00-9:00 pm **WORKSHOP** **Grand Ballroom D&E**

7:00	W-4	Techniques for Preliminary Bacterial Identification and Determination of Suitable Treatment for Infected *In Vitro* Cultured Plants P.M. BUCKLEY
7:30	W-5	Indexing Explants and Cultures to Maintain Clean Stock M.E. KANE
8:00	W-6	The Use of Antibiotics in Plant Tissue Culture G. SECKINGER

TRANSGENIC VEGETABLES IN THE PRODUCT DEVELOPMENT PIPELINE *(P)*

Conveners: Paul Zankowski, Harris Moran Seed Company; Ebe Firoozabady, DNAP

During the past year, genetically engineered tomatoes have been marketed to consumers as the first transgenic plant product. The development of transgenic vegetable products is ongoing along several fronts. These vegetables have improved characteristics for disease resistance, insect resistance, delayed ripening, nutrition, and other properties. The goal of this workshop is to provide a brief summary of transgenic vegetables in the product development pipeline and what the consumer can expect from these products. Representatives from some of the major players in this area will present information and speculation as to the products that might be available in the next few years from transgenic vegetables.

7:00-9:00 pm **WORKSHOP** **Grand Ballroom A-C**

	Introduction (P. Zankowski and E. Firoozabady)
W-7	Commercialization of Ethylene Regulated Fresh Market Tomato J. BEDBROOK, W. Howie, K. Lee, A. Morgan, P. Dunsumuir
W-8	Development of Virus Resistant Cucurbits Through Coat Protein Gene Expression H.D. Quemada, D.M. Tricoli, K.J. Carney, J.F. REYNOLDS, R.Z. Deng, P.F. Russell, J.R. McMaster, M.L. Boeshore, D.W. Groff, K. Hadden, B. Moraghan
W-9	Benefits of Transgenes on the Processing Quality of Tomato E. GREEN
W-10	The Next Generation of High Quality, Genetically Improved Tomatoes J.V. OAKES, C.M. McGuire, C.K. Shewmaker, J.A. Sheehy, R.A. Sanders, W.R. Hiatt, R.E. Sheehy
W-11	Commercialization of Beetle Resistant Potatoes M. HINCHEE
W-12	Enhancing Disease Resistance in Vegetable Crops J.M. JAYNES

CELL DEATH (APOPTOSIS) IN CULTURED CELLS *(I/V)*

Convener: Marcia Loeb, USDA/ARS

Sponsored by Boehringer Mannheim

7:00-9:00 pm **JOINT WORKSHOP** **Majestic Ballroom**

7:00		Introduction (M. Loeb)
7:05	W-13	Apoptosis in an Insect Cell Line: Analogies and Contrasts with Vertebrate Apoptosis R.J. CLEM, J.M. Hardwick, L.K. Miller
7:40	W-14	Nonradioactive Methods for Measuring Cell Death in Cell Populations and Individual Cells A. IMIOLEK

MONDAY, MAY 22

7:00 am-6:00 pm **REGISTRATION** **South Convention Lobby**

DEADLINE—MONDAY, 12:00 NOON
Banquet vouchers MUST be exchanged for banquet ticket if you plan to attend.

ENVIRONMENTAL VARIABLES IN CELLULAR AGING *(V)*

Convener: Thomas Maciag, Holland Labs/American Red Cross

This Symposium will highlight studies being carried out in four different research areas, all of which focus on the elucidation of molecular mechanisms responsible for cellular senescence. The biology of telomere shortening is a fundamental event during cellular senescence. Dr. Chius will present the latest information about the function of the telomere complex in this event. Dr. Passaniti has made a novel observation that B16 melanoma and EHS carcinomas grow poorly as tumors in old mice. He has developed relevant *in vitro* correlates using apoptosis to study this age-dependent phenomena. Dr. Campisi has made numerous contributions to understand human fibroblast senescence. Her recent work has focused on transcriptional controls in the senescent phenotype and the use of cDNA cloning to isolate senescence-induced genes. Genomic cloning strategies have been used to identify chromosomes which are involved in the regulation of cellular senescence. Dr. Hensler will describe the isolation and identification of these regulatory genes.

8:00-10:00 am **SYMPOSIUM** **Majestic Ballroom**

(See abstracts on pages 7A–8A)

8:00		Introduction (T. Maciag)
8:05	V-8	Telomerase Activity is Expressed in Cycling But Not in Quiescent Hematopoietic Progenitors from Adult Human Bone Marrow C.-P. CHIU, V. Dragowski, N.W. Kim, T.E. Thomas, P.M. Lansdorp, C.B. Harley
8:35	V-9	Endothelial Cell Differentiation, Angiogenesis, and the Inhibition of Tumor Growth With Aging A. PASSANITI, R. Pili, C. Yang
9:05	V-10	Transcription Events in Cellular Senescence J. CAMPISI
9:35	V-11	Molecular Genetic Studies of Human Cellular Senescence P.J. HENSLER and O.M. Pereira-Smith

NOVEL INSIGHTS INTO CHEMICAL NEUROTOXICITY *(T)*

Convener: Bellina Veronesi, U.S. EPA
Keynote Speaker: Bellina Veronesi, U.S. EPA

Sponsored by Collaborative Biomedical Products/Becton Dickinson Labware

Pesticides outrank all classes of neurotoxicants in terms of economic importance and environmental risk. Organophosphorous (OP) insecticides are of special concern due to their widespread use. The major biochemical targets of OPs are acetylcholinesterase and neurotoxic esterase, enzymes whose inhibitions can produce lethality or paralysis, respectively. Additionally, OPs at very low doses, can bind to the acetylcholine receptor itself, producing intracellular ionic changes and second messenger events. One of the most perplexing features of OP neurotoxicity is the highly variable biochemical, neuropathological and functional response among species or even strains of the same species that have been exposed to OPs. This phenomenon, known as *interspecies selectivity,* has handicapped rigorous mechanistic investigations into pesticide neurotoxicity. In the Symposium entitled "Novel Insights Into Chemical Neurotoxicity," this phenomenon will be examined in culture using various cellular and subcellular endpoints. Even in culture, mouse and human neuroblastoma cell lines respond differently to test OPs both cytotoxically and

neurotoxically. The experiments presented will collectively explain interspecies selectivity in terms of species differences in inherent cellular metabolism and target enzyme activities, electrophysiological differences at the receptor level and finally second messenger events subsequent to receptor binding. Other studies will describe how mouse and human neuroblastoma cell lines have been used to differentiate between neuropathy-causing OPs and those that only produce lethality. A final speaker will extend the concept of interspecies selectivity to the broader, more human risk relevant area of *selective vulnerability.* Her studies examine the response of different cell types to chemical toxicants. She will also discuss how these differences affect the design of mechanistic studies *in vitro* and technical aspects of using different cell types, including primary neurons, as culture models.

8:00-10:00 am **SYMPOSIUM** **Columbine Room**

(See abstracts on pages 13A–14A)

8:00		Introduction (B. Veronesi)
8:05	T-8	New Insights Into *Interspecies Selectivity* Using Cell Culture Models of Pesticide Neurotoxicity B. VERONESI
8:35	T-9	Technical Aspects of Using Primary Cultures of Nervous Tissue to Investigate Chemical Neurotoxicity H.D. DURHAM
9:05	T-10	Patch Clam Technology in Neurotoxicological Evaluation G. CHRISTOPH
9:35	T-11	*In Vitro* Screens for Esterase-Inhibiting Neurotoxicants M. EHRICH

INTERPLAY OF PLANT CELLS WITH THEIR ENVIRONMENT: ENVIRONMENTAL STRESS (P)

Moderator: Dan Brown, Agriculture Canada

8:00-10:00 am **CONTRIBUTED PAPERS** **Grand Ballroom A-C**

(See abstracts on pages 53A–54A)

8:00	P-1017	Water Deficit Stress-Induced Gene Identification and Transformation in *Pinus* M.A.D. Dias, P. Veeraragavan, J.H. Gould, R.J. NEWTON
8:20	P-1018	Induction of Desiccation Tolerance by ABA and ABA-Analogs in Microspore-Embryos of Canola X. Peng, D.C.W. BROWN, S.R. Abrams, E. Watson, J.A. Webb
8:40	P-1019	The Effects of Various Stresses on Protein Synthesis and mRNA in Soybean Cultures P.S. KAHLON, S.M. Bhatti, and L. Qian
9:00	P-1020	Methyl Jasmonate Induces Cathepsin D Inhibitor in Potato and Tomato Leaves T.-H. ANNIE LIU and D.J. Hannapel
9:20	P-1021	Selection for Resistance to Inhibitors of Polyamine Biosynthetic Enzymes and for Adaptation to High Temperatures in Cotton Cell Cultures R. Saavedra, G.D. Kuehn, G.C. PHILLIPS
9:40		Wrap-up (D. Brown)

REGENERATION AND SELECTION METHODOLOGIES *(P)*

Moderator: D.E. Wedge, Clemson University

8:00-10:00 am **CONTRIBUTED PAPERS** **Grand Ballroom D&E**

(See abstracts on pages 54A–56A)

8:00	P-1022	Callus Induction and Regeneration from Suspension Culture of Garlic, *Allium sativum* J.M. MYERS and P.W. Simon

8:15 P-1023 Comparison of Different Methods of Regeneration for Soybean (*Glycine max* L.) from Mature Seeds and Immature Cotyledons
C.M. BAKER and C.D. Carter

8:30 P-1024 Utilization of Carbohydrates During Organogenesis of *Nicotiana tabacum* L. var. Burley 21
N. AHUJA and N.D. Camper

8:45 P-1025 The Effect of Induction with 2,4-D versus NAA on the Origin, Histology and Normalcy of Pecan Somatic Embryos
A.A.P.M. Rodriguez and H.Y. WETZSTEIN

9:00 P-1026 Responses of *Sassafras albidum* (Nutt.) Nees Explants to IAA and 2,4-D in MS-Culture Media
C.E. BRODERICK

9:15 P-1027 Embryogenic Mango Suspension Cultures Challenged with Culture Filtrate of *Colletotrichum gloeosporioides* Show Enhanced Release of Extracellular Antifungal Proteins
S. JAYASANKAR and R.E. Litz

9:30 P-1028 An *In Vitro* Detection System for *Cornus florida* Calli Resistant to Toxic Metabolites of *Discula destructiva*
D.E. WEDGE, W.V. Baird, F.H. Tainter

9:45 P-1029 Organogenesis and Expression of the Biosynthetic Pathways of Lipid Compounds in *Linum usitatissimum* and *Euphorbia characias*
A. Cunha and M. FERNANDES-FERREIRA

10:00 am-6:00 pm **EXHIBITS AND POSTERS** **Exhibit Hall**

Even Numbered Poster Authors Will Be Present 12:30-1:30 pm
(See list of Posters on pages xxv-A–xxxiv-A)

10:00-10:30 am **COFFEE BREAK** **Exhibit Hall**

TISSUE CULTURE SYSTEMS FOR INVESTIGATING INFECTIOUS AGENTS *(I/V)*

Convener: Gertrude Buehring, University of California-Berkeley

Sponsored by Clonetics Corporation

Despite the great armament of antibiotics and vaccines, infectious diseases remain the leading cause of morbidity and mortality worldwide. For many of the unconquered diseases, our understanding of the causative agent's biology must be vastly expanded before effective strategies for control can be generated. Tissue culture has played a central role in the development of viral vaccines. In addition to its function in mass propagation of microorganisms, tissue culture is increasingly being exploited to study host-parasite relationships, and is often superior to animal models because of the ability to control variables. The speakers in this session will present ingenious *in vitro* models for studying the life cycle and/or pathogenesis of particular infectious agents representing viruses, bacteria, protozoans, and helminths.

10:30 am-12:30 pm **JOINT SYMPOSIUM** **Majestic Ballroom**
(See abstracts on page 2A)

10:30 Introduction (G. Buehring)

10:35 JS-1 Replication of Human Papillomavirus in Differentiating Epithelium *In Vitro*
C. MEYERS and L.A. Laimins

11:05 JS-2 Epithelial/Endothelial Bilayer Tissue Culture Model for the Study of Bacterial Pathogenesis
F.D. QUINN, K.A. Birkness, J.H. Bartlett, E.H. White

11:35	JS-3	Axenic Culture of Malarial Parasites W. TRAGER
12:05	JS-4	Culture Methods for the River Blindness Parasite, *Onchocerca volvulus.* M.S. CUPP, T. Lehman, E.W. Cupp

PCR METHODS IN TOXICOLOGY *(T)*

Convener: Oliver Flint, Bristol Myers Squibb

Sponsored by Roche Molecular Systems

Genetic variability and gene expression can now be readily evaluated by PCR (polymerase chain reaction). The cytochrome P450 family of xenobiotic metabolizing enzymes was originally characterized by studies of reaction kinetics, metabolic specificity and immunoassay. PCR has provided a new and very precise tool for identifying cytochrome P450 genes and, by using the reverse transcriptase enzyme, the expression of P450 mRNA. More traditional methods, such as the use of fluorescent probes, were able to identify broad classes of P450 enzyme. PCR, in contrast, permits the precise identification of individual genes both in intact tissues and in cultured cells, expanding our knowledge of the maintenance of the differentiated state under *in vitro* conditions.

10:30 am-12:30 pm **SYMPOSIUM** **Columbine Room**

(See abstracts on pages 14A–15A)

10:30		Introduction (O. Flint)
10:35	T-12	Xenobiotic-mediated Induction of Cytochrome P450 Gene Expression in Primary and Transformed Rat Hepatocyte Cultures: Differences in Expression and Induction Potential as Ascertained by RT-PCR. J.S. SIDHU, F.M. Farin, C.J. Omiecinski
11:05	T-13	Detection of Human Extrahepatic Cytochrome P450 (CYP) Expression Using RT-PCR. F.M. FARIN, M.R. Andersen, C.J. Omiecinski
11:35	T-14	Human Genetic Diversity in Carcinogen Metabolism: Probing Molecular Variation by Polymerase Chain Reaction D.A. BELL, A. Hirvonen, M. Watson

IN VITRO FOREST TREE STRATEGIES *(P)*

Conveners: David Ellis, University of Wisconsin; Neville Arnold, Agriculture Canada

The *in vitro* manipulation of forest trees has presented some unique challenges to researchers. These challenges have contributed to an increased understanding of limitations with woody plant tissue culture, as well as the development of tissue culture systems to overcome some of these limitations. Somatic embryogenesis is an example of one of these systems, which has been important not only for propagation, but also for molecular and biochemical studies. Although somatic embryogenesis offers many advantages, the inability to propagate most mature trees remains a large obstacle. An understanding of the physiological, biochemical, and molecular changes associated with the phase shift from juvenile to mature tissues has yielded considerable information useful for the propagation of mature genotypes. These studies have been focused on perennial crops yet have yielded information applicable to the *in vitro* manipulation of all plants.

10:30 am-12:30 pm **SYMPOSIUM** **Grand Ballroom A-C**

(See abstracts on pages 18A–19A)

10:30	P-6	Strategies for Dealing With Limitations of Somatic Embryogenesis in Hardwood Trees S.A. MERKLE

10:55	P-7	Somatic Embryogenesis in *Picea abies:* Morphological and Biochemical Characterization of Various Developmental Stages S. VON ARNOLD, H. Mo, U. Egertsdotter
11:20	P-8	Cellular, Biochemical and Molecular Bases of Stable Maturation-Related Characteristics W.P. HACKETT
11:45	P-9	Immature Embryo Culture of *Quercus alba* K.S. GRUMBINE and N.D. Camper
12:00	P-10	Lower Oxygen and Somatic Embryogenesis of Loblolly Pine (*Pinus taeda* L.) F.H. HUANG and X.Y. Li
12:15	P-11	Imaging of Fluorescent-stained Somatic and Zygotic Pine Embryos with Laser Scanning Confocal Microscopy R. NAGMANI, A. Kakani, A.M. Diner, G. Brown, V. Sapra, G.C. Sharma

12:30-1:30 pm **POSTER PRESENTATION** **Exhibit Hall**

Even Numbered Poster Authors Present
(See list of Posters on pages xxv-A–xxxiv-A)

THREE DIMENSIONAL TISSUE CULTURE IN NASA BIOREACTOR SYSTEMS *(V)*

Conveners: Neal R. Pellis, NASA-Johnson Space Center;
J. Milburn Jessup, M.D. Anderson Cancer Center

Sponsored by NASA-Johnson Space Center

The *in vitro* manipulation of forest trees has presented some unique challenges to researchers. These challenges have contributed to an increased understanding of limitations with woody plant tissue culture, as well as the development of tissue culture systems to overcome some of these limitations. Somatic embryogenesis is an example of one of these systems, which has been important not only for propagation, but also for molecular and biochemical studies. Although somatic embryogenesis offers many advantages, the inability to propagate most mature trees remains a large obstacle. An understanding of the physiological, biochemical, and molecular changes associated with the phase shift from juvenile to mature tissues has yielded considerable information useful for the propagation of mature genotypes. These studies have been focused on perennial crops, yet have yielded information applicable to the *in vitro* manipulation of all plants.

2:30-4:30 pm **SYMPOSIUM** **Majestic Ballroom**

(See abstracts on pages 8A–10A)

2:30		Introduction (N.R. Pellis)
2:35	V-12	Low Shear Stress of NASA Rotating Wall Vessel (RWV) Increases CO_2 and Acid Production While Supporting Differentiation in Three-Dimensional Cultures J.M. JESSUP, A. Nachman, R.D. Ford
2:50	V-13	Microgravity-Suppressed Peripheral Blood Mononuclear Cell (PBMC) Locomotion Is Restored by Iron-Transferrin Supplementation R.R. PIZZINI and N.R. Pellis
3:05	V-14	Three Dimensional Multicellular Systems *In Vitro*: NASA Bioreactor and Other Techniques L. MARGOLIS, W. Fitzgerald, N. Amichai, B. Baibakov, S. Glushakova, J. Zimmerberg
3:20	V-15	Simulated Microgravity Enhances Extracellular Matrix Protein Expression in Cultured PC12 Pheochromocytoma Cells J. LIU, D.L. Galvan, B.R. Unsworth, P.I. Lelkes

3:35	V-16	Human Renal Epithelial Cells in Culture Differentiate Under Simulated Microgravity T.G. HAMMOND, D.L. Galvin, T.J. Goodwin, P.I. Lelkes
3:50	V-17	Microgravity Enhances Tissue-Specific Neuroendocrine Differentiation in Cocultures of Rat Adrenal Medullary Parenchymal and Endothelial Cells D.L. GALVAN, B.R. Unsworth, T.J. Goodwin, J. Liu, P.I. Lelkes
4:05	V-18	Brief Exposure to Simulated Microgravity Affects Tyrosine Phosphorylation in PC12 Pheochromocytoma Cells D.L. GALVAN, B.R. Unsworth, T.J. Goodwin, P.I. Lelkes
4:20	V-19	Increased Cytotoxicity of Bleomycin When Used In Conjunction With Electroporation R. HELLER, M. Jaroszeski, R. Perrott, J. Becker, H. Arango, P. Satyaswaroop, R. Gilbert
4:35	V-20	To Be Announced KERBEL

CELLULAR AND MOLECULAR TOXICOLOGY *(T)*

Moderator: Marque Todd, Xenometrix, Inc.

2:30-4:30 pm **CONTRIBUTED PAPERS** **Columbine Room**

(See abstracts on pages 40A–41A)

2:30	T-1001	Detecting the Transcriptional Responses to Genotoxins in Mammalian Cells With and Without Exogenous Bioactivation M.D. TODD, P. Gee, S.B. Farr
2:50	T-1002	Atypical Cytochrome P-450 Induction Profiles at the mRNA and Enzyme Level in Glomerular Mesangial Cells A.R. PARRISH, R.C. Bowes III, M.A. Steinberg, K.L. Willett, W. Zhao, S.H. Safe, K.S. Ramos
3:10	T-1003	The Cytotoxicity of 4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone on Pancreatic Duct Cell Cultures M.K. REDDY
3:30	T-1004	Retinol Stimulation of Clara Cell-Antigen Expression in an Epithelial Stem-Cell Line (M3E3/C3) of Syrian Hamster Lung M. EMURA, A. Ochiai, G. Singh, I. Hilger, S.L. Katyal, D.L. Dungworth
3:50	T-1005	A New Assay for Toxicity of Alloplastic Materials in the Urinary Tract: Preliminary Results With Bladder Catheters J.V. Harney, C.E. MOTHERSILL, D.M. Murphy

THE DISCOVERY AND PRODUCTION OF PHARMACEUTICALS FROM PLANT TISSUE CULTURES *(P)*

Convener: Michael Horn, Mycogen Plant Sciences

It has long been known that plant cell cultures are capable of synthesizing secondary compounds of pharmaceutical and commercial value. The process of putting plant cells into culture induces the expression of numerous isozymes which are rarely, if ever, found together in any organ of the plant at any point in its life cycle. Hence, new secondary compounds are frequently synthesized by plant cell cultures, albeit usually in quite low amounts. Moreover, culturing plant cells as heterotrophic, photoautotrophic, embryogenic, and 'organized' induces a myriad of changes in the isozymal complement with the expected result being new secondary compounds found in each 'culture form'.

The detection, characterization, and testing of these novel compounds are of potentially great importance as the biodiversity of the Earth's flora continues to decline. This session will focus on what is known about secondary compounds in cultured plant cells including synthesis, biotransformation, and commercialization. First, Dr. Masanaru Misawa will give an overview of the state of the art in this area. Then, Dr. David Ellis will give a lecture on the history of Taxol and the production of this valuable anti-

cancer compound *in vitro.* Subsequent talks will focus on the production of ginkgolides from cultures of *Ginkgo biloba,* and the effect of different gases in the culture headspace on Taxol production.

2:30-4:30 pm **SYMPOSIUM** **Grand Ballroom A-C**

(See abstracts on pages 19A–20A)

2:30	P-12	Plant Cell Culture: An Alternative for Production of Pharmaceuticals M. MISAWA
3:10	P-13	Taxol—The Science and History of an Anti-Cancer Compound from U.S. Forests D. ELLIS
3:50	P-14	Taxol Productivity of Suspension Cultures of *Taxus cuspidata* Exposed to Defined Headspace Gas Concentrations N. MIRJALILI and J.C. Linden
4:05	P-15	Use of Rotating Wall Vessel (RWV) for Study of Plant Cell Culture X. SUN and J.C. Linden
4:20	P-16	*In Vitro* Culture of Ginkgo N.D. CAMPER, D.E. Wedge, R.J. Keese, A. Depew

CELL COMMUNICATION IN INVERTEBRATES *(I)*

Convener: Kathleen Horwath, SUNY at Binghampton

2:30-4:30 pm **SYMPOSIUM** **Vail Room**

(See abstracts on pages 22A–23A)

2:30		Introduction (K. Horwath)
2:35	I-7	A Single Second Messenger Mediating Opposing Growth Cone Behaviors S.B. KATER, T.B. Kuhn, A. Shibata, M.V. Wright, C.V. Williams
3:05	I-8	Analysis of Neurogenic Signalling in Cultured Cell Lines from *Drosophila melanogaster* M.A.T. MUSKAVITCH, T.R. Parody, S.B. Shepard, M. Vaskova
3:35	I-9	2OHE Induced Neuronal Differentiation *In Vitro* (Insects) R. LEVINE
4:05	I-10	Molecular Analysis of Ecdysone Action in Insect Cells S.R. PALLI, T. Ladd, B. Cook, S.S. Sohi, A. Retnakaran

5:00-6:00 pm **HAPPY HOUR** **Exhibit Hall**

HOW TO INTRODUCE ELEMENTARY SCHOOL STUDENTS TO *IN VITRO* BIOLOGY

Convener: Helene N. Guttman, Agricultural Research Service/USDA

Dannette Ward of Monsanto Co., St. Louis, will conduct the workshop. Dr. Ward has many years of experience doing "show and tell" demonstrations about *in vitro* biology for elementary school students and their teachers. She will share with us general principles for addressing this type of audience, and the type of materials that should be brought to the school for a successful demonstration that ***captures*** and ***holds*** the interest of this audience. She will do a model demonstration for us and have available at the meeting handouts that she provides to the teachers so that they can continue with further *inexpensive* teaching modules for the students.

One role of scientists and their professional societies is to assist in transmitting the excitement of science to youngsters since they represent our future. We expect this workshop to be the first of a series; however, a measure of its success will be two-fold: attend the workshop, *and,* then, visit an elementary school and show the students and teachers what you have learned.

7:00-9:00 pm **EDUCATION COURSE** **Silver Room**

TUESDAY, MAY 23

7:00 am-6:00 pm **REGISTRATION** **South Convention Lobby**

METHODS AND MARKERS FOR EUKARYOTIC TRANSFORMATION AND THE FATE OF DNA *(P/T/V)*

Conveners: Ray Shillito, Ciba Corporation; John Harbell, Microbiological Associates, Inc.

Co-sponsored by Ciba Corporation and ICI Seeds, Inc.

Transgenic technology has become a vital part of the study of genes, and their expression and interaction in all higher eukaryotic organisms. Techniques have been developed for introducing genes and monitoring their activity. However, there has been little interaction between those working in different organisms. Cross fertilization of ideas and insights will lead to new approaches to transformation. This session will therefore bring together information on transformation methods, marker technology, and behavior of inserted DNA sequences in different eukaryotes, and promote discussion between those working on related issues in different organisms.

8:00-10:00 am **JOINT SYMPOSIUM** **Grand Ballroom A-C**

(See abstracts on pages 3A–4A)

8:00	JS-5	Real Time Analysis of Transcription Using *In Vivo* Reporter Gene Technology S.A. KAY
8:30	JS-6	Particle Bombardment Technology for Gene Transfer into Plant and Mammalian Systems N.-S. YANG
9:00	JS-7	Factors Affecting Stable Transformation via Micro-projectile Bombardment Using Immature Corn Embryos A.D. BAILEY, E. Brambila, S.G. DeWald
9:20	JS-8	DNA Transformation Using Electrically Charged Tungsten Microelectrodes T.J. HARRINGTON and E. Aamodt
9:40	JS-9	Factors Enhancing *Agrobacterium*-Mediated Transformation of Peanut (*Arachis hypogaea* L.) M. EGNIN, A. Mora, C.S. Prakash

10:00 am-5:00 pm **EXHIBITS AND POSTERS** **Exhibit Hall**

Odd Numbered Poster Authors Will Be Present 12:30-1:30 pm

(See list of Posters on pages xxv-A–xxxiv-A)

10:00-10:30 am **COFFEE BREAK** **Exhibit Hall**

FROM NORMAL TO NEOPLASTIC: CANCER DEVELOPMENT *IN VITRO (V)*

Convener: Alda Vidrich, Cedars Sinai Medical Center

Neoplastic transformation is a multistage process involving events that can be temporally distinguished as well as the accumulation of multiple changes in gene expression. A number of cell culture systems have been particularly instrumental in the elucidation of mechanisms leading to the evolution of the transformed cell. This Symposium reviews our current understanding of the biological, biochemical, and genetic changes that contribute to the development of the malignant cell. This knowledge now provides an opportunity to identify agents that may disrupt the process of carcinogenesis.

10:30 am-12:30 pm **SYMPOSIUM** **Majestic Ballroom**

(See abstracts on page 11A)

10:30		Introduction (A. Vidrich)
10:35	V-21	Neoplastic Transformation of Human Fibroblasts in Culture—A Multi-stepped Process J.J. MCCORMICK and V.M. Maher
11:05	V-22	Defects in Cell Cycle Control and Differentiation in Multistage Cancer of Mouse Epidermal Cells M. KULESZ-MARTIN
11:35	V-23	Induction, Progression, and Prevention of Carcinogenesis in Cultured Respiratory Epithelial Cells V.E. STEELE

MEDIATORS OF INFLAMMATION AND IMMUNOTOXICOLOGY *(T)*

Convener: Pat Dimond, PerSeptive Biosystems
Keynote Speaker: Michael I. Luster, NIEHS

Transgenic technology has become a vital part of the study of genes, and their expression and interaction in all higher eukaryotic organisms. Techniques have been developed for introducing genes and monitoring their activity. However, there has been little interaction between those working in different organisms. Cross fertilization of ideas and insights will lead to new approaches to transformation. This session will therefore bring together information on transformation methods, marker technology, and behavior of inserted DNA sequences in different eukaryotes, and promote discussion between those working on related issues in different organisms.

10:30 am-12:30 pm **SYMPOSIUM** **Columbine Room**

(See abstracts on pages 15A–16A)

10:30		Introduction (P. Dimond)
10:35	T-15	The Role of Cytokines in Chemical Toxicity M.I. LUSTER
11:05	T-16	Air Pollutants and Alveolar Macrophage (AM) Function: Relevance of *In Vitro* Exposures to *In Vivo* Effects M.J.K. SELGRADE
11:35	T-17	Direct Effects of 2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin (TCDD) on B-lymphocyte Function: Mechanistic Studies and Comparative Studies Between Mouse and Man M.P. HOLSAPPLE
12:05	T-18	The Role of Interleukin-10 in the Induction of Immune Suppression by UV Exposure S.E. ULLRICH

TRANSFORMATION CHALLENGES FOR RECALCITRANT CROPS: RECENT BREAKTHROUGHS *(P)*

Convener: Maud Hinchee, Monsanto Co.

Co-sponsored by Monsanto Company

This workshop explores some recent developments in plant transformation. The focus will be on the development of transformation systems as well as on relatively new transformation methods. The techniques to develop a routine transformation system in three problematic plant species will be discussed. In addition, two relatively recent developments in transformation methods will also be described. The intent of this workshop is to elucidate the thought processes and experimental strategies necessary to develop new transformation technology.

10:30 am-12:30 pm **WORKSHOP** **Grand Ballroom A-C**

(See abstracts on pages 27A–28A)

Introduction (M. Hinchee)

W-15 Transformation of Grape (*Vitis vinifera* L.)
R. SCORZA, J.M. Cordts, D.J. Gray, D.W. Ramming, R.L. Emershad

W-16 Pea Transformation
A. MORGAN

W-17 High Efficiency Transformation of Regeneration of Transgenic Sweetpotato Plants
C.S. PRAKASH, Q. Zheng, A. Porobo Dessai

W-18 Plastid Transformation: A New Tool for Basic Science and for Biotechnological Applications
P. MALIGA

W-19 *Agrobacterium*-mediated Gene Transfer to Rice (*Oryza sativa* L.)
J.R. ROUT, M.P. Gordon, W.J. Lucas, E.W. Nester

INSECT CELLS IN CULTURE *(I)*

Moderator: Cynthia Goodman, USDA/ARS

10:30 am-12:30 pm **CONTRIBUTED PAPERS** **Vail Room**

(See abstracts on pages 85A–86A)

10:30 I-1001 Insect Midgut Cells in Culture: A Typical Stem Cell System
M.J. LOEB and R.S. Hakim

10:50 I-1002 Characteristics of Midgut-Derived Insect Cell Lines
C.L. GOODMAN and A.H. McIntosh

11:10 I-1003 Baculovirus *Ac*MNPV Induces Apoptosis in an Insect Midgut Cell Line
S.R. PALLI, G.F. Caputo, A.J. Brownright, S.S. Sohi

11:30 I-1004 Interaction Between Cell Adhesion and Apoptosis Pathways and its Role in the Cellular Immune Response in Insects
L.L. PECH and M.R. Strand

11:50 I-1005 Identification of Insect Cell Lines by DAF
A.H. MCINTOSH, J.J. Grasela, R.L. Matteri

12:30-1:30 pm **POSTER PRESENTATION** **Exhibit Hall**

Odd Numbered Poster Authors Present
(See list of Posters on pages xxv-A–xxxiv-A)

BIOTECHNOLOGY—GROWTH OF CELLS IN BIOREACTORS *(V/T)*

Convener: Kathy Allen, IDEC Pharmaceuticals Corp.

Sponsored by PerSeptive Biosystems, Inc.

The use of cultured cells to manufacture pharmaceutical products is a complex and highly-regulated process that requires expertise in numerous areas of cell culture research including heterohybridoma culture, genetic engineering, cell line characterization, media adaptation, and many others. A variety of cells are used for production, including plasmid-transfected CHO cells, baculovirus-transfected insect cells, and E. coli. The cells are genetically altered by transfection to include the gene DNA sequences of the desired product. The transfected cells used for producing the proteins or antibodies can be cultured in various types of bioreactors, from the low-volume hollow-fiber bioreactors of 25-mL to large-scale production in stirred tanks holding 10,000 L. Cellular metabolism is maintained by manipulating culture medium components and environmental culture conditions to optimize cell growth and product expression.

In this Symposium, an overview of the entire process, as well as specific phases, of developing a cell line engineered to perform a desired function are described.

2:30-5:30 pm — **JOINT SYMPOSIUM** — **Grand Ballroom A-C**

(See abstracts on pages 4A–5A)

2:30		Introduction (K. Allen)
2:35	JS-10	Biotechnology Products from Animal Cells A.S. LUBINIECKI
3:20	JS-11	Genetics and Molecular Biology of Gene Transfer, Amplification and Expression in Recombinant Chinese Hamster Ovary Cells F.M. WURM
3:55	JS-12	Genetic and Phenotypic Traits Observed During Development, Implementation and Monitoring of Large-Scale Mammalian Cell Culture Manufacturing Processes S.R. ADAMSON
4:30	JS-13	Monitoring Monoclonal Antibody Production W.G. ROBEY, J. Brackett, K. Cousineau, G. Gall, B. Peterson, A. Annapragada, H. Wang

TRANSFORMATION CHALLENGES FOR RECALCITRANT CROPS: FOCUS ON SOYBEAN *(P)*

Convener: Ted Klein, Dupont Agricultural Products

Co-sponsored by Dupont Agricultural Products, American Soybean Association

The production of transgenic soybean was first reported in 1988. Although progress in the transformation of this species has occurred since then, there is still a need for a generally applicable and efficient gene transfer system. This workshop will provide a forum for discussion of recent progress in this field.

2:30-4:30 pm — **WORKSHOP** — **Majestic Ballroom**

(See abstracts on pages 28A–29A)

W-20	The Components of Variation Associated with *Agrobacterium*-Mediated Transformation of Soybean T.E. CLEMENTE, B.J. La Valle, D.A. Kasten, K.K. Seehra, D.L. Broyles, P.E. Hunter, R.J. Rozman, D.C. Ward, A.R. Howe, M.A. Hinchee
W-21	Possible Factors Affecting Fertility of Soybean Plants from Transgenic Embryogenic Cultures W.A. PARROTT, C.N. Stewart, M. Anis
W-22	Establishment of a Regional Soybean Tissue Culture and Genetic Engineering Center R.D. DINKINS, R.S. Torisky, R. Di, G.B. Collins
W-23	Cotyledonary Node Explants of Northern-Adapted Soybeans as Targets for *Agrobacterium*-Mediated Transformation P.A. DONALDSON, D.H. Simmonds, H. Voldeng
W-24	Transformation and Transformation-competence in Embryogenic Tissue of Soybean J.J. FINER, E.R. Santarem, H.N. Trick

5:00-6:00 pm — **SIVB BUSINESS MEETING** — **Silver Room**

All Members Are Urged to Attend

TUESDAY, MAY 23

ATTENTION POSTER PRESENTERS
All Posters Must Be Removed from Exhibit Hall
By 5:00 pm Tuesday, May 23

AWARDS BANQUET • 7:00 pm
Seating is Limited
Admittance to Banquet by Advance
Ticket Holders Only

WEDNESDAY, MAY 24

7:00 am-12:00 pm **REGISTRATION** **South Convention Lobby**

CANCER BIOLOGY *IN VITRO (V)*

Moderator: Carmel Mothersill, Dublin Institute of Technology

8:00-10:30 am **CONTRIBUTED PAPERS** **Denver Room**

(See abstracts on pages 32A–33A)

8:00 V-1007 Long-term Alteration in the Expression of Cell Cycle Control and Signal Transduction Genes Following Exposure of Human Urothelial cultures to Gamma Radiation
C. MOTHERSILL, J. Harney, F. Lyng, C. Seymour, K. Parsons, D. Murphy

8:20 V-1008 Effects of Vitamin D and its Analogs on Breast Carcinoma Cells
R.R. MEHTA, R.G. Mehta, T.K. Das Gupta

8:40 V-1009 Experimental Down-Regulation of c-myc Oncogene-Induced Transformation in Mammary Epithelial Cells: Effect of Brassinin Derivatives
N.T. TELANG, S. Inoue, R.G. Mehta, R.M. Moriarty, H.L. Bradlow, M.P. Osborne

9:00 V-1011 The Development of a Model of Cancer Initiation and Progression Using Conditionally Immortalised Colonic Mucosal Cells
R.H. WHITEHEAD, J. Weinstock, J.L. Joseph

BIOTECHNOLOGY: MODELS & METHODS *(T)*

Moderator: Patricia Chulada, NIEHS

8:15-10:30 am **CONTRIBUTED PAPERS** **Silver Room**

(See abstracts on pages 41A–42A)

8:15 T-1006 Selective Inhibition of Murine Prostaglandin Synthase 1 or 2 by NSAIDs Using Mammalian Cell Lines Retrovirally Infected With Murine Prostaglandin Synthase cDNAs
P.C. CHULADA and R. Langenbach

8:35 T-1007 Stripping and reconstitution of HLA Class I Associated Peptide for Generation of Antigen-specific CD8 + T-lymphocytes
M.V. PESHWA and W.C.A. van Schooten

8:55 T-1008 Development of a Fluorescence Based Amino Acid Analysis System Suitable for Analysis of Tissue Culture Media and Cell Culture Broth
C. VAN WANDELEN, S.A. Cohen, J.T. Kubiak

9:15 T-1009 Serum-Free Production of Rotaviruses
S. GOULD, D. DiStefano, D. Robinson

9:35 T-1010 Identification and Characterization of the Extracellular Matrix Molecule Restrictin
J.J. HEMPERLY, R.L. Ackley, R.A. Reid

9:55 T-1011 Hematotoxic Effects of Chemotherapeutic Drugs Assessed Using Three-Dimensional Bone Marrow Cultures
J. SAN ROMÁN, V. Kamali, B. Sibanda, J.M. Gee, B.A. Naughton

LIQUID MEDIUM IN PLANT *IN VITRO* CULTURE *(P)*

Conveners: Indra Vasil, University of Florida; Robert Levin, Osmotek Ltd.

Sponsored by Life Technologies

This workshop will address the use of cluster cultures, mechanical separation of cluster culture, problems of contamination, filter sterilization of media, and use of rafts.

8:00-10:30 am **WORKSHOP** **Grand Ballroom A-C**

(See abstracts on page 30A)

Introduction (I. Vasil/R. Levin)

W-25 Improvement of Regeneration of Nontransgenic and Transgenic Plant Tissues Using a Concentrate Liquid Medium
J.-J. LIN, R.M. Fike, N. Assad-Garcia

W-26 Advantages of Microporous Membranes for Plant Tissue Culture on Liquid Media
J.W. ADELBERG and R.E. Young

W-27 Plant Micropropagation in Bioreactor Cultures
M. ZIV

W-28 Liquid Culture as a Route for High Efficiency Micropropagation
A.A. WATAD, V. Gaba, Y. Alper, R. Levin

MATRIGEL® MATRIX-BASED CULTURE SYSTEMS FOR PRIMARY HEPATOCYTES

Conveners: Brigitta Tadmor, Collaborative Biomedical Products/Becton Dickinson Labware

Sponsored by Collaborative Biomedical Products/Becton Dickinson Labware

In order to study cellular processes in physiologically relevant manner, the microenvironment *in vitro* must replicate certain characteristics of the microenvironment *in vivo.* Maintaining a cell's biological function *in vitro* often requires culture conditions that allow for cellular interactions with extracellular matrix, soluble factors (e.g., cytokines or hormones) and homo- or hetero-typic interactions between cells. Hence, the construction of an *in vitro* system for the culture of cells, particularly primary cells, requires optimization of its key components, such as extracellular matrix and cytokines.

During the first part of this workshop, the impact of culture conditions on cell morphology and cell function will be illustrated on the example of hepatocytes and other epithelial cells. During the second and third part, MATRIGEL® Matrix-based systems for the culture of differentiated hepatocytes will be discussed. Methodology to determine cell morphology (e.g., TEM and SEM) and cell function (e.g., expression of specific cytochromes and liver-specific transcription factors) will be presented.

8:00-10:30 am **WORKSHOP** **Grand Ballroom D&E**

(Abstracts will be distributed at the workshop)

TOM COLLINS, Collaborative Biomedical Products/Becton Dickinson Labware
PAULA FLUHERLY, Collaborative Biomedical Products/Becton Dickinson Labware
STEPHEN FARMER, Boston University School of Medicine

VERTEBRATE/INVERTEBRATE POSTERS

SUNDAY, MAY 21	MONDAY, MAY 22	TUESDAY, MAY 23
10:00 am-6:00 pm	10:00 am-6:00 pm	10:00 am-5:00 pm

POSTER SESSION

Posters Mounted Saturday, May 20, 3:00-6:00 pm
Posters must be removed from Exhibit Hall by 5:00 pm, Tuesday, May 23

Authors will be present at their posters the following days and times:

SUNDAY, MAY 21	MONDAY, MAY 22	TUESDAY, MAY 23
Even Authors Present 12:30-1:30 pm Odd Authors Present 4:30-5:30 pm	Even Authors Present 12:30-1:30 pm	Odd Authors Present 12:30-1:30 pm

BIOTECHNOLOGY

V-1012 A New Culture Method of Three-Dimensionally Reconstituted Multicellular Mass Utilizing Cotton-Gauze
T. TAKEZAWA and K. Yoshizato

V-1013 Archival Storage and Analytical Display of Culture Media
E.K. WHITE, T. Cuffel, R.G. Ham

V-1014 Viral Inactivation of Serum with Ultra Violet (UV) Irradiation
V.H. WILLIAMSON

CANCER BIOLOGY

V-1015 The Effect of Platinum Pharmacokinetics on the Growth of Low-Dose-Rate Radiation Resistant Peripheral Blood Lymphocytes
S.L. SCHNEIDER, M. Szekeresova, M. DeGregorio

V-1016 Abstract has been withdrawn.

V-1017 Melatonin Modulates the Inhibitory Action of Some Chlorinated Acids on Intercellular Communication
S.G. BENANE, C.F. Blackman, D.E. House

CELLULAR IMMUNOLOGY

V-1018 Isolation and Characterization of Monoclonal Antibodies to Surface Molecules of a Compaction-Defective Mutant of the Multicell Tumor Spheroid Phenotype
S.N. GARCIA, P. Pineda, L.A. Jordan, L.S. Armstrong, A.O. Martinez

V-1019 Analysis of Somatic Cell Hybrids Between MTS^+ and MTS^- Cell Lines for Expression of Multicell Tumor Spheroid (MTS^+) Phenotype
M.L. UBINAS, J. Martinez, J. Pizarro, L.S. Armstrong, A.O. Martinez

V-1020 A New Monoclonal Antibody 5G7 Reacting with Human Leukocytes
E. DIMITROVA, H. Taskov, M. Nikolova, A. Pashov

CELLULAR MODEL

V-1021 Human Hydatidiform Mole in Culture: A Multi-Nucleated Trophoblast Cell Line
D. THOMPSON, G.E. Sarto

V-1022 Growth Factors Produced by the LA7 Rat Mammary Tumor Cell Line Stimulate Proliferation of Mouse Mammary Epithelial Cells
U.K. EHMANN, J.T. De Vries, M.S.C. Chen, A.A. Adamos

V-1023 Caloric Restriction *In Vitro*: Role of Serum on Cultured Adipocytes from Rats Fed Ad Libitum (AL) and Calorically Restricted (CR) Diets
B.S. HASS, R.W. Hart, N.A. Littlefield, A. Turturro

V-1024 Transport Mechanism of Histamine Receptor Type 2 (H_2) Antagonists in Caco-2 Cells
H.H. FARRISH, S.B. Yanni, L.-S. Gan, P.-H. Hsyu

VERTEBRATE/INVERTEBRATE POSTERS

DIFFERENTIATED CELLS

V-1025 Serum-free Media for Growth of Seven Mammalian Kidney Cell Types
J.A. DARNER, P. Miller, F. Simon, B.A. Van der Haegen

V-1026 Characterization of Skeletal Muscle Atrophy Induced in Simulated Microgravity Culture Systems
D. BROWN, K.I. Clark, N.R. Pellis, T.J. Goodwin

V-1027 Antagonistic Actions of Triiodothyronine and Dexamethasone on the Differentiation of Cultured Adult Human Jaw Bone Osteoblasts
C. Guerriero, D. De Santis, P. Gotte, P.F. Nocini, U. ARMATO

EXTRACELLULAR MATRIX

V-1028 *In Vitro* Production of Basement Membrane Extracellular Matrix by Human Umbilical Venous Endothelial Cells
E.J. ROEMER, M. Spektor, S.R. Simon

V-1029 An Improved Method for Ascorbate Supplementation of R22 Cells in Culture for *In Vitro* Biosynthesis of Interstitial Extracellular Matrix
E.J. ROEMER, M. Spektor, S.R. Simon

V-1030 Divalent Cations and Assembly of the FGF Receptor Complex
M. KAN, F. Wang, W.L. McKeehan

INVERTEBRATE

I-1006 *In Vitro* Studies with the Corpora Allata of *Manduca sexta*
B.G. UNNI

SILENT

V-1031 Retinoblastoma Derived Growth Factor Stimulation of DNA Synthesis in Human Retinal Pigment Epithelial Cells in Culture
J.F. TARSIO

V-1032 A Novel Serum-Free Medium for the Cultivation of Vero Cells on Microcarriers
Z. CHEN, C. Xiao, H. Liu, B. Wu, X. Jia, Z. Huang

V-1033 Failure of CFTR Plasma Membrane Targeting of CFTR in a CF Pancreatic Duct Cell Line
C. Chemin-Thomas, C. Gonindard, C. Devaux, O. Guy-Crotte, C. Figarella, E. HOLLANDE

V-1010 Human Virus Detection Using Cells Immortalised by Oncogenes
J.B. CLARKE, H. Moulsdale, J. Golding, B. Griffiths

I-1007 Inhibitory Effect of Niclex on Glycogen of Tape Worm (Neokrimia singhia)
M.R. SIVA SAI KUMARI

TOXICOLOGY POSTERS

SUNDAY, MAY 21	MONDAY, MAY 22	TUESDAY, MAY 23
10:00 am-6:00 pm	10:00 am-6:00 pm	10:00 am-5:00 pm

POSTER SESSION

Posters Mounted Saturday, May 20, 3:00-6:00 pm
Posters must be removed from Exhibit Hall by 5:00 pm, Tuesday, May 23

Authors will be present at their posters the following days and times:

SUNDAY, MAY 21	MONDAY, MAY 22	TUESDAY, MAY 23
Even Authors Present 12:30-1:30 pm Odd Authors Present 4:30-5:30 pm	Even Authors Present 12:30-1:30 pm	Odd Authors Present 12:30-1:30 pm

BIOTECHNOLOGY METHODS

T-1012 IL-4 Production Using Macroporous Microcarriers
B. LUNDGREN, J. Shirokaze, K. Yanagida, K. Shudo, K. Konomoto, K. Kamiya, K. Sagara

T-1013 Subculture Method for Large Scale Cell Culture Using Macroporous Microcarrier
B. LUNDGREN, K. Kamiya, K. Yanagida, J. Shirokaze

T-1014 A Novel Method for the Analysis of Amino Acids in Cell Culture Media
J.M. KUBIAK, C. Van Wandelen, S.F. Gorfien

T-1015 Sensitivity of Isoenzyme Analysis for the Detection of Cell Line Cross-Contamination
R.W. NIMS, A.P. Shoemaker, M.A. Bauernschub, J.W. Harbell

T-1016 Cells on Rotating Fibers
R. CLYDE

T-1017 Photoregulation, Purification and Application of B-phycoerythrin from *Porphiridium cruentum*
K. MINKOVA, A. Tchernov, E. Dimitrova, A. Mihova

CELLULAR MODELS IN TOXICOLOGY: TEST SYSTEMS

T-1018 Production of an *In Vitro* Reconstituted Skin Using Human Neonatal Foreskin Keratinocytes (HFK) in Combination with the Dermal Substrate AlloDerm®
E.S. GRIFFEY and S.A. Livesey

T-1019 *In Vitro* Cytotoxicity Testing of Potentially Active Anti-HIV Drugs with Cultured Cells
F.A. BARILE, D. Hopkinson, P. Scheiner

T-1020 Agar Diffusion Cytolysis and Aqueous pH: A Classification Algorithm of Two *In Vitro* Tests for *In Vivo* Ocular Hazard Categorization
D.A. LASKA, W.P. Hoffman, J.T. Reboulet

T-1021 Rabbit Corneal Epithelial Cell Lines as an *In Vitro* Alternative Model for Evaluating the Efficacy and Cellular Toxicity of Drugs: I. Immortalization and Preliminary Characterizations
C. YAO, D. Wampler, D. Grimm, K. Hall, D. Shade, D. Crouch, J. Veltman, R. Hackett

T-1022 Immortalized Lens Epithelial Cells as an *In Vitro* Model for the Efficacy and Cellular Toxicity Evaluations of Ocular Drugs: I. Immortalization of Cells and Preliminary Characterizations
D. Wampler, D. Grimm, Guo-Tung Xu, D. Shade, R. Hackett, C. YAO

T-1023 A Human (HepG2) Cell Line Model for Cadmium Toxicity Studies
P.F. DEHN, C.M. White, D.E. Conners, G. Shipkey, T.A. Cumbo

T-1024 Comparison of pH 6.70 SHE and Balb/c-3T3 Transformation Assays to Ames and Rodent Bioassay Results
R.M. BRAUNINGER, G.A. Kerckaert, R.A. LeBoeuf

T-1025 Communication By Keyword: Enhanced Storage, Retrieval and Dissemination of Information About *In Vitro* Technologies
D.J. HUGGINS

TOXICOLOGY POSTERS

CELLULAR MODELS IN TOXICOLOGY: MECHANISMS

T-1026 Effects of External Ligands on Interaction of $CdCl_2$ with Cell Lines of Different Tissue Origins
J.T. JONES, D.E. Carter, H.E. Laird II

T-1027 *In Vitro* Endoreduplication of Chromosomes by Organomercurials in CHO Cells
A. WILSON, L. Carleton, E. Alauddin, T.S. Kochhar

T-1028 Phosgene-induced Calcium Changes in Pulmonary Artery Endothelial Cells
R.J. Werrlein, S.D. Kirby, J. MADREN-WHALLEY

T-1029 2,2′ Dichlorodiethyl Sulfide (Sulfur Mustard, SM) Causes Cleavage of Human Lymphocyte DNA Poly (ADP-Ribose) Polymerase Inhibitors (PADPRPI) Alter the DNA Patterns
H.L. MEIER and C.B. Millard

T-1030 DNA Damage Caused by Influenza Virus-Single Cell Electrophoresis Assay
M.V. RAMANA, Y.L. Ahuja, G. Sharma

SILENT

T-1031 The Role of Glutathione in Protecting Against Menadione-induced Cytotoxicity in Platelets Isolated from Rats
Y.S. Cho, K.S. Park, J.Y. Lee, M.J. Kim, J.H. CHUNG

T-1032 Comparative Heavy Metal Cytotoxicity to Established Fish Cell Lines
H. SEGNER

T-1033 Immortalization and Depolarizing Conditions Modulate c-Fos Expression in Retinal Cell Cultures
G.M. SEIGEL

PLANT POSTERS

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MICROPROPAGATION

P-1030 Use of Clones in a Sugarbeet Improvement Program
L. PANELLA and C. Rivera Smith

P-1031 Micropropagation of Cowpea *(Vigna unguiculata)* Through Shoot Tip Multiplication
J.M. AL-KHAYRI, T.E. Morelock, E.J. Anderson

P-1032 Micropropagation of Agarita, *Berberis trifoliata*
F. MOLINAR, JR., W.A. Mackay, M.M. Wall

P-1033 Micropropagation of Banana Through Synseed Technology
A.K. SUDHA VANI and G.M. Reddy

P-1034 Inflorescence Development From *In Vitro* Node Cultures of Switchgrass
K.S. ALEXANDROVA, P.D. Denchev, B.V. Conger

P-1035 Use of an Acoustic Window in Ultrasonic Production of Nutrient Mist for Tissue Cultures
M.J. CORRELL, P.J. Weathers, D. Walcerz, J. Czarnecki, M. Gibson, R. Owen

P-1036 *In Vitro* Propagation of *Litsea Cubeba* (Lours) Pers. (Lauraceae)
A.A. MAO, A. Wetten, P.D.S. Caligari

P-1037 Cryopreservation of *Arachis glabrata* Benth. Shoot Tips by Vitrification
L.E. TOWILL

P-1038 Velvetleaf *(Abutilon theophrasti):* The Effects of Thidiazuron on *In Vitro* Culture of Seedlings and Seedling-derived Leaf and Hypocotyl Explants
C.A. Wiley and D.A. STEEN

P-1099 Tissue Culture Studies on the Nodal Explants of *Psidium Guajava* (Guava)
Z.M. SIDDIQUI and S.A. Farooq

MICROBE INTERACTIONS

P-1039 Isolation of Rhodococcus Metabolites with Plant-Cell Regulation Activity by XAD-Adsorbent Resins
A.C. González, J.L. IBAVE, J.C. López

P-1040 Detection and Characterization of Bacterial Contaminants of Micropropagated Strawberry
P. TANPRASERT

P-1041 Bacterial Contaminants of *Corylus In Vitro* Cultures
B.M. REED, J. Mentzer, P. Tanprasert, X. Yu, P. Buckley

P-1042 Somatic Embryogenesis and Plantlet Regeneration in Rice Callus
S. SANGAM and P.B. Kavi Kishor

REGENERATION

P-1043 Growth Regulator and Genotype Effects on Somatic Embryogenesis from Sugarbeet Callus
C.J. TSAI and J.W. Saunders

PLANT POSTERS

P-1044 Increase in Somatic Embryogenesis from *Dactylis glomerata* L. Leaf Cultures by Silver Thiosulfate and Hypobaric Conditions
A.I. KUKLIN, C.E. Sams, B.V. Conger

P-1045 Somatic Embryogenesis in Pigeon Pea (*Cajanus Cajan* L.)
S.R. ANBAZHAGAN

P-1046 High Frequency Shoot Formation and Plant Regeneration from Mature Embryos of *Syzygium cuminii*
S.K. ROY and M.S. Islam

P-1047 Effect of Silver Nitrate on Callus and Regeneration in Cotton Species
Z.-S. KE and J.McD. Stewart

P-1048 Asymmetric Somatic Hybridization via Protoplast Fusion in Peanuts
Z. Li, A. Xing, M. CHENG, R.L. Jarret, R.N. Pittman, J.W. Demski

P-1049 *In Vitro* Shoot Multiplication of Carnation Axillary Buds and Nodes
M.S. BRAR, J.M. Al-Khayri, G.L. Klingaman

P-1050 The Effects of Ethylene and Ethylene Inhibitors on White Spruce Embryogenic Tissue Maintenance and Somatic Embryo Maturation
L. KONG and E.C. Yeung

P-1051 Propagation of *Rauvolfia serpentina* By *In Vitro* Shoot Tip Culture
S.K. ROY, M.Z. Hossain, N. Alam

P-1052 Genotypic Basis for Multiple Shoot Induction from De-embryonated Cotyledons of Groundnut
A. SABITHA and G.M. Reddy

P-1053 Induction of Enhanced Plant Regeneration from Callus Cultures of Some *Indica* Rice Varieties
J.S. SANDHU, M.S. Gill, S.S. Gosal

P-1054 *In Vitro* Regeneration and Protoplast Culture Studies in Mungbean (*Vigna radiata* [L.] Wilczek)
D.T. SELVI, N.M. Ramaswamy, S. Sukumar, S.R. Sree Rangasamy

P-1055 Factors Affecting Organogenesis and Somatic Embryogenesis in Eggplant
P. SHARMA and M.V. Rajam

P-1056 *In Vitro* and *In Vivo* Multiplication of Virus-Free "Spunta" Potato
R.A. SHIBLI, A.M. Abu-Ein, M.M. Ajlouni

P-1057 Induction of Direct Multiple Shoots from Cotyledons and Meristems of *Gossypium Hirsutum* L.
S. TRIPATHY and G.M. Reddy

P-1058 Induction of Embryogenesis by Anther Culture of Pigeonpea
P. VIJAYAKUMARI and S. Narasimha Chary

P-1059 Callus Induction, Plant Regeneration and Somatic Embryogenesis in Primary Trisomics (2n+1) of Indica Rice *Oryza Sativa* L.
N. FATIMA and S.Y. Anwar

P-1060 Plant Regeneration from Immature Embryos of 48 Elite CIMMYT Bread Wheats
S. FENNELL, N. Bohorova, M. van Ginkel, J. Crossa, D. Hoisington

P-1061 Isolation of Fattyacid Desaturase Genes (Fad 2 and Fad 3) from Genomic Library of *Arachis Hypogaea* L.
E.C. KANTH and G.M. Reddy

P-1062 Molecular Studies on *In Vitro* Flowering in *Arachis hypogaea* L.
T.A. KUMAR and G.M. Reddy

P-1063 Selection for Improved Agronomic Value in Pigeonpea Somaclones Regenerated from Cotyledonary Explants
P. LATHA, J.P. Moss, K.K. Sharma, J.K. Bhalla

P-1064 High Frequency Callusing and Green Plant Regeneration from Anthers of Indica Rice
G.V. LAXMI and G.M. Reddy

P-1065 Effect of Thidiazuron on Regeneration from 'Half-Seed Explants' of *Capsicum annuum* L.
M.L. Binzel, N. Sankhla, D. Sankhla, T.D. DAVIS, S. Joshi

P-1066 Leaves Roots and Suspension Cultured Cells of Rice (*Oryza Sativa* L.) and its Genomic Polymorphism Analysed Using RAPD
P.H. BAO, S. Granata, E. Cuzzoni, C. Giordani, S. Castiglione, G. Wang, F. Sala

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P-1067 Multiple Virus Eradication from Potato
C. ZAPATA, J.C. Miller, R.H. Smith

P-1068 Anther Culture Studies from Salt Tolerant Cultivars of Indica Rice
S.K. ANITHA and G.M. Reddy

P-1069 Genetic Analysis of Salinity Tolerance in Rice
K.J. REDDY and G.M. Reddy

P-1070 Salt-Responses in *Oryza sativa* Seedlings: Role of Calcium and Gibberelic Acid in Salt Toxicity
S. SANGAM and P.B. Kavi Kishor

P-1071 The Influence of Osmoticum on Protoplast Yields of Selected *Eucalyptus dunnii* Maid. Clones
M.E.C. Graca, H.G. Hughes, S.D. REID

P-1138 *In Vitro* Propagation of Mangosteen (*Garcinia mangostana* L.) From Shoot Cultures
R. Aliudin and M.N. NORMAH

SECONDARY PRODUCTS

P-1072 Expression of Anthocyanins in Bilberry and Huckleberry Callus Cultures
D.L. MADHAVI, M.A.L. Smith, R. Rogers

P-1073 Anthycyanins in *Ocimum basilicum* cv. Purpurascens *In Vitro*
D.L. MADHAVI, M.A.L. Smith, S. Juthangkoon

P1074 Physical Microenvironmental Effects on Anthocyanin Production in Cell Cultures of *Ajuga pyramidalis* 'Metallica Crispa'
S. JUTHANGKOON

P-1075 Triterpenoid Composition of *In Vitro* Tissues of Maritime Pine (Pinus pinaster Ait)
A.C.P. Dias and M. FERNANDES-FERREIRA

P-1076 Synthesis and Accumulation of Essential Oils in *In Vitro* Regenerated Shoots and *Calli* of *Chamaemelum nobile*
L.P.C. Santos-Gomes and M. FERNANDES-FERREIRA

P-1077 Enhancing Production of Artemisinin in Transformed Roots of *Artemisia annua*
P. WEATHERS, T. Smith, D. Hemmanvanh, E. Follansbee, J. Ryan, R. Cheetham

P-1142 Comparisons of *Artemisia annua* Root Cultures, and *Nephrolepsis exaltata* Whole Plant Cultures in a Newly Designed Nutrient-mist Bioreactor with Conventional Methods
C.S. BUER, M.J. Towler, T.C. Smith, P.J. Weathers, D. Walcerz

TRANSFORMATION

P-1078 Regeneration and Transformation in Sunflower (*Helianthus annuus* L.) Mature Cotyledons
C.M. BAKER and C.D. Carter

P-1079 Effect of Donor Plant and Culture Factors on Transient Gene Expression in Alfalfa Following Microprojectile Bombardment
L.-N. TIAN, D.C.W. Brown, J. Webb

P-1080 Plant Regeneration and *Agrobacterium*-mediated Transfer of *ROlC* Gene in *Salpiglossis sinuata* L.
C.W. LEE and L. Wang

P-1081 Protoplast-mediated Transformation of Peanut *(Arachis hypogaea)* for Virus Resistance
Z. Li, A. Xing, M. CHENG, R.L. Jarret, and J.W. Demski

P-1082 Transformation of Elite Maize Inbreds by Microprojectile Bombardment of Type I Callus
J. DAWSON, E. Dunder, N. Palekar, J. Suttie

P-1083 High Frequency Co-Transformation of Embryogenic Peanut Cultures
H.D. Wilde, Z.V. Magbanua, W.A. PARROTT

P-1084 *In Vitro* Regeneration and Potential Transformation of Peanut (*Arachis hypogaea* L. cv. Okrun)
J. PONSAMUEL, D.V. Huhman, B.G. Cassidy, R.S. Nelson, D. Post-Beittenmiller

P-1085 Genetic Transformation of Seashore Mallow by *Agrobacterium tumefaciens*
J.D. RAO, D.M. Seliskar, J.L. Gallagher

P-1086 Transient Expression of CAT and GUS Activities in Maize Embryos and in Germinating Pollen
N. TSENGWA, J.A. Saunders, R. Patel, M.S. McIntosh

P-1087 Optimization of Particle Bombardment Conditions for Long Term Stable Expression Using GUS Gene In Wheat
W. CHUNG WANG and D. Marshall

P-1088 Effect of Timentin for Controlling *Agrobacterium tumefaciens* Following Cocultivation on Select Plant Species
T.W. ZIMMERMAN

P-1089 Rice Transformation Using *Agrobacterium* and the Shoot Apex
S.H. PARK and R.H. Smith

P-1090 Insect Chitinase-mediated Resistance to Tobacco Budworm *(Heliothis virescens)* in Transgenic Tobacco Plants
X. DING, L. Johnson, F. White, B. Gopalakrishnan, K. Kramer, S. Muthukrishnan

P-1091 Transformation of *Sorghum bicolor* L.
L.-S. KO and R.H. Smith

P-1092 Transformation of *Solanum brevidens* Using *Agrobacterium tumefaciens*
T.-H. ANNIE LIU, L.C. Stephens, D.J. Hannapel

P-1093 Transformation of *Euphorbia lathyris* by *Agrobacterium rhizogenes*
E. Follansbee, R. CHEETHAM, P. Weathers

P-1094 Manipulation of the Maize Meristem for Transformation
M. ROSS, L. Church, V. Phillips, P. Troy, W. Gordon-Kamm

P-1095 Germline Transformation of Maize Using Shoot Multiplication to Enlarge Chimeric Sectors
K. LOWE, G. Hoerster, M. Ross, W. Gordon-Kamm

P-1096 Characterization of Transgene Insertion and Expression in a Glufosinate-Resistant Maize Line
T.M. SPENCER, L.C. Wilson, R.J. Daines, P. Julstrom, R. Mumm, C.E. Flick

P-1097 CIMMYT Efforts Towards the Production of Transgenic Tropical Maize With Enhanced Insect Resistance
N.E. BOHOROVA, B. Luna, R.M. Brito, A.M. Maciel, L. Diaz, M.E. Ramos, D.A. Hoisington

P-1098 HMGCoA Reductase Gene: A Unique RFLP Marker for the Varietal Identification of *Oryza sativa* L.
D. ROY, I. Chaudhuri, R.K. Chaudhuri

SILENT

P-1100 Micropropagation of *Milicia excelsa*
Y. LIN, M.R. Wagner, J.R. Cobbinah

P-1101 Effect of Inoculation with Four Strains of the Beneficial Bacteria *Azospirillum* Spp. on Proteins and Peroxidase Content in Cowpea *(Vigna unguiculata)* Calli
L. ALCRAZ-MELÉNDEZ, S. Real-Cosío, Y. Bashan

P-1102 Improved Embryogenesis and Plants Regeneration from Isolated Microspore Culture of Wheat (*Triticum aestivum* L.)
T. HU and K.J. Kasha

P-1103 Protoplast Isolation and Fusion in Two Seedless Orange Cultivars
R.F. GHAZVINI

P-1104 Unexpected Morphogenetic Responses Induced by Auxins Alone in *Mammillaria saangelensis*, A Severely Endangered Cacti
T. MARIN and A. Rubluo

PLANT POSTERS

P-1105 Embryogenesis from Callus Culture of Immature Zygotic Embryo of *Commiphora wightii*
A. KUMARI

P-1106 Direct Somatic Embryogenesis from Immature Zygotic Embryos of *Commiphora wightii*
A. KUMARI

P-1107 Regeneration Ability via Somatic Embryogenesis in Pigeon pea (*Cajanus cajan* [L] Millsp)
P. KAUR and J.K. Bhalla

P-1108 Factors Affecting the Formation of Embryogenic Callus and Embryogenesis from Undeveloped Ovules of Valencia and Washington Navel Orange
R.F. GHAZVINI

P-1109 Genetic Manipulation of Certain Grain Legumes Using Tissue Culture Methods
D. RAO and A. Ramulu

P-1110 Utilisation of Thidiazuron (TDZ) for Development of Effective Regeneration System in *Lycopersicon esculentum* Mill.
T. ZAKIR, E. Iankova, L. Sthereva, N. Zagorska

P-1111 Biological Control of Crown Gall on Peach
M. Pérez-Cavala, J.M. Haro-Hernández, M. Ramos-Parra, O. VAZQUEZ-MARTINEZ

P-1112 *In Vitro* Clonal Propagation of Guava Tree (*Psidium quajava* L.)
O. VAZQUEZ-MARTINEZ, E. Pérez Molphe-Balch

P-1113 Effects of Preparation and Storage on Gelled Medium Water Status and *In Vitro* Growth of Cranberry *(Vaccinium macrocarpon)* and Grape (*Vitis vinifera* L.) Cultures
J.U. TOLEDO, M.A.L. Smith, L. A. Spomer, D. Madhavi

P-1114 Asymmetric Intergeneric Somatic Hybrid Plants Between *Lycopersicon esculentum* x *L. pennellii* (+) *Solanum melongena*
V.M. SAMOYLOV and K.C. Sink

P-1115 A Protoplast-to-Plant Protocol for Mulberry Trees
Y. SAHOO, S.K. Pattnaik, P.K. Chand

P-1116 *In Vitro* Culture of Tomato Hybrids (*Lycopersicon esculentum* Mill.)
T.C. NARAYNASWAMY and N.M. Ramaswamy

P-1117 Comparison of Taxol Productions Between In Situ and *In Vitro* Tissues
C.Y. HU, G. Sharma, M. Sahni, N.C. Vance, D. Ravindranath

P-1118 Promoter Type and Cell Age Influences on Marker Gene Expression in Suspension Cultured Cells Following Particle Bombardment
S.M. DETHIER ROGERS, T. Ueda, R.J. Newton, K.M. Dias

P-1119 Regeneration of Soybean From Bulgarian Genotypes
D. SEKULICHKA, G. Kosturkova

P-1120 Somatic Embryogenesis and Plant Regeneration for Efficient Gene Manipulation in Higher Plants
G.M. REDDY

P-1121 *In Vitro* Tumor Formation on *Simondsia chinensis* infected by *Agrobacterium tumefaciens*
O. VAZQUEZ-MARTINEZ, J.J. Moreno-Hernández Duque, L.L. Valera-Montero

P-1122 Present Status of Clonal Propagation of Elite Plants of Oil Palm (*Elaeis quineensis* Jacq.) by Tissue Culture of Immature Inflorescences in Costa Rica
N.M. GUZMAN

P-1123 Preliminary Studies to Determine the Micropropagation Potential of *Pistacia chinensis*
D.E. DUNN and J.C. Cole

P-1124 Clonal Propagation of Four Medicinal Plants: *Ocimum americanum* L. (hoary basil), *O. basilicum* L. (sweet basil), *O. gratissimum* L. (shrubby basil) and *O. sanctum* L. (sacred basil) Through *In Vitro* Culture of Axillary Vegetative Buds
SK. PETTNAIK, Y. Sahoo, P.K. Chand

P-1125 Clonal Propagation and Comparative Analysis of Free Radical Scavanging Enzymes in *In Vitro* and *In Vivo* Tissues of *Gmelina arborea* Roxb.
V. RAMESH KANNAN, Y.T. Jasrai

P-1126 Regeneration on Ability of Herbicide Tolerant Variants, R_1 Selfed and Back Crossed Progenies
N. SIMEONOVA, N. Nedkovska, N. Tineva

PLANT POSTERS

P-1127 Selection of Tobacco Somaclonal Variants Resistant to Diseases and Herbicides
N. SIMEONOVA, M. Nedkovska, N. Zagorska

P-1128 Salt Tolerance in Cell Lines of Pistachio (Pistachio vera)
A. SHEIBANI and T.A. Villiers

P-1129 Effect of Some Phenolic Compounds on the Senescence of Intact Primary Leaf of Bean (*Phaseolus vulgaris* L.) with Particular Reference to the Activities of Certain Hydrolytic and Oxidative Enzymes
V.S. NISHAKARA CHARY

P-1130 Role of Hydroxyproline in Salt Tolerance of Rice
V.A. CHAUHAN and G. Prathapasenan

P-1131 Induction and Repair of UV-C Induced DNA Single-Strand Breaks in Repair of Deficient Strain of Chlamydomonas reinhardtii
E.D. BLAGOEVA, L.M. Stoilov, S.G. Chankova

P-1132 Effect of 2,4-Dichlorophenoxyacetic acid and Zeatin on Somatic Embryogenesis of Flax (*Linum usitatissimum* L.)
A. Cunha and M. FERNANDES-FERREIRA

P-1133 Factors Effecting *In Vitro* Flowering In Brassica juncea L. Czern and Cross
K. SRINIVASA CHARY and S.A. Forook

P-1134 Protein Characterization During Morphogenesis in Pigeonpea (*Cajanus cajan* [L.] *Millsp.*)
K. SREENIVASU and S.N. Chary

P-1135 Ureide Catabolism in N_2 Fixing Pigeon pea
D.S. REDDY, G. Reddy, H. Polasa

P-1136 *In Vitro* Tuberization in Sweet Potato (*Ipomoea batatas* L. Lam)
J.E.B.P. PINTO and S.A. Figueiredo

P-1137 Micropropagation of Onion Through *In Vitro* Bulblet Formation
J.E.B.P. PINTO and B.M. Rodrigues

P-1139 *In Vitro* Propagation of *Acacia holocersia* - Forest Tree
K.K. REDDY, P. Bhaskar, K. Subash

P-1140 *In Vitro* Selection for Salt Tolerance: Changes in Activity and Induction of Specific Isozymes During Salinity Stress in *Oryza sativa* Cultivars
A.V. RATNAM and G.M. Reddy

P-1141 *In Vitro* Techniques for Selection of Anthio Tolerant Cell Lines in *Cicer arietinum* (L.)
R.A. CHINNALA

PS-1 The Cellular Immune Response of Insects: In Vitro Approaches to the Study of Antiviral and Antiparasitic Defense Mechanisms
MICHAEL STRAND, Department of Entomology, University of Wisconsin, Madison, WI 53706

Circulating blood cells (hemocytes) play an essential role in defending invertebrates against internal pathogens and parasites. Metazoan parasites and other large invaders are usually killed by encapsulation; a process in which certain classes of hemocytes attach and spread across the surface of the foreign target, forming a multilayered sheath of cells. Not surprisingly, parasites have evolved several strategies to avoid the cellular defense responses of their hosts. Key to deciphering these interactions are in vitro studies using primary cultures of hemocytes and related cell lines. Here I summarize progress in this area, emphasizing studies on insect systems and their associated parasites. Capsule formation by insects requires a coordinated response by specific classes of hemocytes with strong intercellular adhesion mediated in part by an Arg-Gly-Asp (RGD)-dependent adhesion mechanism. To avoid encapsulation several parasites introduce factors into their host that disrupt intercellular adhesion or selectively eliminate hemocytes involved in nonself recognition. For one large group of insect parasites, these events are mediated by symbiotic polydnaviruses. These viruses replicate in the adult stage of the female parasite and are introduced into hosts when the parasite lays its eggs. How these viruses disrupt the host cellular defense system and its implications for antiviral defense strategies in insects are discussed.

PS-2 CONTROL OF CELL GROWTH AND DIFFERENTIATION BY EXTRACELLULAR MATRIX. Donald E. Ingber, Depts. of Pathology & Surgery, Children's Hospital & Harvard Medical School, Boston, MA 02115.

Our past work has revealed that extracellular matrix (ECM) molecules regulate cell sensitivity to soluble mitogens and thereby switch cells between growth and differentiation in the local tissue microenvironment. Our research efforts are now concentrated in two major areas: 1) analysis of the biochemical mechanism by which binding of ECM to cell surface integrin receptors activates intracellular chemical signaling pathways and induces transcription of growth and differentiation-specific genes, and 2) dissection of the biomechanical mechanism by which transferring mechanical forces between ECM and integrin receptors alters cell, cytoskeletal and nuclear form as well as cellular biochemistry. The first approach is based on the finding that integrin clustering results in activation of multiple chemical signaling pathways (e.g., Na+/H+ antiporter, protein tyrosine phosphorylation, inositol lipid metabolism) that were previously thought only to be coupled to growth factor receptors. The second approach is based on studies with three dimensional "tensegrity" cell models (literally constructed from sticks and elastic string) which suggest that many of the effects of ECM on cell shape can be based largely on biomechanical considerations. By combining cell biological methods with engineering approaches, we have recently been able to confirm that integrins mediate mechanical signal transfer across the cell surface and that living cells respond to mechanical stresses as if they were tensegrity structures. We also have demonstrated that cytoskeletal tension-dependent changes in cell shape play a critical role in terms of switching cells between growth and differentiation programs. Studies are underway to characterize this biomechanical signaling system and to understand both the biochemical and biomechanical basis of cell form determination. Results of these studies should have widespread implications for control of morphogenesis and may facilitate the development of new therapeutic modalities that may be used for the treatment conditions, such as cancer, which depend on uncontrolled cell growth and differentiation for their development.

PS-3 Do Plants Take Aspirin?
ILYA RASKIN
AgBiotech Center, Rutgers Univ., New Brunswick, NJ 08903 USA

Salicylic acid (SA) is a likely endogenous signal in the development of systemic acquired resistance in dicotyledonous plants for the following reasons:

- SA levels increase locally and systemically following inoculation
- SA increases are sufficient for the induction of PR proteins and resistance
- Environmental, developmental, and genetic changes in resistance and PR protein expression correlate with changes in tissue SA
- SA moves in the plant
- Blocking SA accumulation blocks SAR

β-*O*-D-GlucosylSA (GSA) and volatile methyl salicylate are the major metabolites of SA in tobacco. GSA accumulates rapidly in tobacco leaves treated with SA or in the immediate vicinity of TMV-induced lesions. GSA is formed by the action of a UDPglucose:SA glucosyltransferase, which is induced by the high levels of SA present in TMV-inoculated tobacco leaves. The synthesis of SA from benzoic acid in tobacco leaves is catalyzed by a benzoic acid 2-hydroxylase (BA2H). BA2H acts as a Cyt P450 monooxygenase which specifically catalyzes the 2-hydroxylation of BA to SA using atmospheric oxygen. Antibodies against SU2, a soluble Cyt P450 from *Streptomyces griseolus*, depleted the BA2H activity from soluble protein fractions and immunoprecipitated a protein putatively identified as BA2H. BA2H was also activated *in vitro* by hydrogen peroxide. Labeling endogenously produced SA with $^{18}O_2$ demonstrated that systemic increases in SA in healthy tissues can be at least partially explained by SA transport from the TMV-inoculated leaf.

PS-4 Developing Valid *In Vitro* Alternatives for Toxicology and Pharmacology. Eugene Elmore, 28612 Camelback Road, Trabuco Canyon, CA 92679.

Recent advances in cellular and molecular biology have resulted in a greater understanding of regulatory mechanisms for cellular growth, homeostasis, differentiation, and cellular and tissue specific function. Many of these advances have resulted from the increasing availability of human cells and tissues and the development of characterized and defined culture media that permit cells to grow or express differentiated function similar to that observed *in vivo*. New molecular biomarkers provide the specific tools required to define and characterize the role of cell to cell interactions and the changes in specific genetic expression that occur during normal growth and development. Molecular biomarkers also provide insights as to the potential toxicity and efficacy of therapeutic or environmental agents. With these advances comes the potential for relative *in vitro* assessment of the effects of chemical or drug exposure on human biological responses. While much of the historical information has been obtained using cultures of specific cells from the tissues being studied, future *in vitro* systems must be designed to accommodate the functional aspects of the *in vivo* systems that are being modeled. Only by understanding the regulatory interactions of cells and tissues in their environment can we predict their role *in vivo*.

JS-1 Replication of Human Papillomavirus in Differentiating Epithelium *In Vitro*

Craig Meyers* and Laimonis A. Laiminș†, *Dept. of Microbiology and Immunology, The Milton Hershey Medical Center, Penn State University, Hershey, PA 17033 and †Dept. of Microbiology and Immunology, Northwestern University, Chicago, IL 60611.

The specific tropism of papillomaviruses for keratinizing epithelium and host specificity has been an impediment for developing a system to study virus-cell interaction in a natural situation. Research on papillomaviruses has greatly benefited from the improvements made with organotypic (raft) culture system techniques. Recently we reported the development of a cell culture system that allows for the first time the *in vitro* biosynthesis of papillomavirus particles from a continuous cell line. The block to virion production was attributed to an incomplete differentiation program in raft culture. By the addition of the phorbol ester TPA to the raft culture media virion biosynthesis was induced concomitant with a more complete differentiation program. This differentiation induction response was seen in raft cultures of all cell lines tested; including lines derived from invasive cervical carcinomas, high grade cervical neoplasias, and low grade cervical neoplasias. Using this unique system we have characterized the viral gene expression patterns during the complete life cycle of papillomaviruses.

Further investigations are demonstrating the facility of the raft system to be used: a) to studying the mechanisms controlling the proliferation and differentiation of epithelium; b) to propagate different papillomavirus types *in vitro*; c) to studying the mechanisms of carcinogenesis; and d) to identify carcinogens (both epigenetic and genotoxic) in an *in vitro* human tissue system.

JS-2 **Epithelial/Endothelial Bilayer Tissue Culture Model for the Study of Bacterial Pathogenesis. Frederick D. Quinn, Kristin A. Birkness, Jeanine H. Bartlett, and Elizabeth H. White, Centers for Disease Control and Prevention, Atlanta, GA 30333**

A tissue culture bilayer system has been developed as a model to study the mechanisms of attachment, invasion, transcytosis, or intercellular passage through tight junctions by several bacterial pathogens, including: *Neisseria meningitidis*, *Mycobacterium tuberculosis*, and *M. avium*. The model incorporates epithelial and endothelial cell layers separated by a microporous membrane. This construction makes it possible to observe and quantify the attachment and passage of bacteria through the multiple layers and to study the mechanisms by which they make this passage. This process approximates activities that must occur during an infection as the microbe makes its way from the mucosal surface through the epithelial cells and into the vascular system. A major strength of this system is that it is generally adaptable to a wide variety of microbial pathogens and can be modified by substituting any physiologically relevant epithelial, endothelial or lymphatic eukaryotic cell for the component layers. Using this model we have identified a mutant of *Neisseria meningitidis* that passes through a HeclB/HMEC-1 bilayer 1,000 - 10,000-fold less efficiently than wild-type strains. We also have examined routes of infection of *M. tuberculosis* and *M. avium* as the bacteria pass through a human pneumocyte/human alveolar vascular endothelial cell bilayer. When fresh human macrophages are added to the surface of this particular model the macrophages apparently localize themselves on the pneumocyte surface, within the pneumocyte layer, and below the endothelial layer in the vascular space. With the addition of the macrophages, the physiology of this system more closely approximates that of the human alveolus, and thus lung pathogens can be examined more accurately. In general, the makeup of the bilayer system using cells of human origin and its reproducibility give it advantages over animal and primary organ culture models. The added complexity of multiple layers allowing cell-to-cell communication make it a more realistic human tissue model than standard cell monolayers.

JS-3 Axenic Culture of Malarial Parasites

W. TRAGER, Rockefeller Univ., New York, NY 10021

Continuous cultivation of Plasmodium falciparum, most important of the human malarial parasites, was first reported by me and J.B. Jensen in 1976. The method depends on multiplication of the erythrocytic stages of the parasite in human erythrocytes maintained in a tissue culture medium, with daily change of medium, at 37-38°C, and in an atmosphere with 3-5% CO_2 and O_2 at 17% or less. This method made it possible to carry on research with this major human pathogen in any well-equipped laboratory; for many kinds of work it was no longer necessary to maintain the parasites in the Aotus or Saimiri monkeys which are the only suitable experimental animals. As a result, a great deal has been learned about the cell and molecular biology of P. falciparum, about its genetics and biochemistry, about its immunology and its susceptibility and resistance to drugs. The information acquired has already proved useful for the development of experimental vaccines, especially for the erythrocytic stages. Of particular interest have been recent studies on the export of proteins from the parasite into the erythrocyte cytosol and even to the erythrocyte membrane. Some of these proteins have a role in pathogenesis and in immune evasion, but the function of most of them is as yet unknown. In further pursuit of the details of the host parasite relationship, I and my colleagues, J. Williams and G.S. Gill, have now obtained true axenic development of the parasites. Merozoites, the invasive forms, develop extracellularly through the asexual cycle to again form merozoites 45 hr later. With present conditions, only about 2% of the merozoites complete the cycle.

JS-4 Culture methods for the River Blindness parasite, *Onchocerca volvulus*. Mary S. Cupp, Tovi Lehman and E.W. Cupp. Department of Veterinary Science, University of Arizona, Tucson AZ 85721 and Entomology Branch, Division of Parasitic Diseases, Centers for Disease Control, Chamblee GA.

River blindness is the common term for human onchocerciasis, a disease which is caused by the filarial parasite, *Onchocerca volvulus*. More than 100 million persons are at risk of infection, primarily in Africa and Central and South America. Although damage to the eye is the most devastating pathology, microfilariae are found most commonly in skin where uptake by blood-feeding black flies occurs. The normal life-cycle of this parasite occurs in black flies for the first three immature stages of development and in humans for further development to reproductive adults. Axenic culture of the complete life cycle has not been accomplished but maintenance and limited growth and development of several stages have been obtained. Cell-conditioned medium from the monkey kidney cell line $LLCMK_2$ and decreased oxygen tension enhanced growth and differentiation of *Onchocerca* spp. third-stage larvae (L_3s) to the fourth-stage (L_4). This culture technique has allowed the screening of potential filaricidal drugs and studies of vertebrate host/parasite interactions. A Matrigel stratum was used to study microfilarial migration within the invertebrate host. Examination of isolated host factors by this method showed that microfilariae follow chemical cues of the host to reach their anatomical site of development.

JS-5

Real Time Analysis of Transcription Using *In Vivo* Reporter Gene Technology

Steve A. Kay
NSF Center for Biological Timing, Department of Biology, University of Virginia, Charlottesville, VA 22903.

Luciferase as a genetic reporter combined with ultra low-light imaging systems offers a means to study dynamic fluctuations in transcriptional activity in real time. In addition, these methods provide a non-invasive way to study these changes as well as allow the development of genetic screens based on subtle differences in transcriptional activity.

The recent development of modified forms of Green Fluorescent Protein (GFP) means that a wide range of luminescent and fluorescent reporter genes are available for monitoring transcriptional regulation or transformation events in living cells, tissues and organisms.

Recent advances in the development and application of these technologies will be discussed for both plant and animal systems.

JS-6

Particle Bombardment Technology for Gene Transfer into Plant and Mammalian Systems. NING-SUN YANG, Agracetus, Inc., 8520 University Green, Middleton, WI 53562

Among the various gene transfer techniques, the gene gun-mediated delivery method has unique features which make it applicable to a wide spectrum of experimental systems including plants, insects, fish, and mammals. It is effective on a wide range of target tissues, including *in vitro*, *ex vivo*, and *in vivo* systems. Since delivery is accomplished by a physical means, many of the constraints associated with conventional gene transfer methodology have been minimized or eliminated completely. Thus, in the case of plant systems, genotype and host-cell specificity, culture-induced variation, or difficulties with regeneration from single cells and fully dedifferentiated tissue, and in the case of mammalian systems, diverse cell types regardless of their origin, no longer present insurmountable barriers for the creation of transgenic tissues and organisms. Initially, the gene gun method was used to demonstrate delivery of foreign DNA into plant cells, resulting in the creation of transgenic elite varieties of soybean, cotton, rice, bean, corn, woody species, and others. This technology probably made the most significant contribution to the field of plant genetic engineering during the past eight years, revolutionizing plant gene transfer and creating a generic strategy for production of transgenic crops. For many experimental animal systems, *in vivo* gene transfer to various somatic tissues, as well as *ex vivo* gene transfer to tissue explants and their derivative primary cultures was desired, but has been technically difficult to achieve using existing technology. The gene gun methods have been evaluated on a number of these systems, and hand-held devices were developed to facilitate particle-mediated gene transfer to targeted tissues of live animals. Particle-mediated gene transfer to skin, liver, muscle, kidney, pancreas, and other somatic tissues of live animals was demonstrated in several mammalian species, as well as *ex vivo* gene transfer to freshly isolated tissue explants. These technical advancements provided new approaches for studying *in vivo* and *ex vivo* transgene expression in a variety of mammalian systems. Potential applications of the gene gun technology to genetic therapy and genetic immunization are being actively evaluated. In one approach for cancer gene therapy, various transgenic cytokines, locally produced via *in vivo* and *in situ* gene gun delivery of skin tissues overlying subcutaneous tumors were shown to activate immune responses resulting in antitumor activity in mice. By targeting mouse epidermis *in vivo* with several cellular and viral gene constructions, several researchers have demonstrated production of transgenic foreign proteins which elicit humoral and/or cytotoxic cellular immune responses, suggesting that the gene gun technology may also have multiple applications to gene therapy, genetic immunization, and cancer vaccination.

JS-7

Factors Affecting Stable Transformation via Microprojectile Bombardment using Immature Corn Embryos. ANITA D. BAILEY, Eduardo Brambila, Stephen G. DeWald. Novel Genetics Dept., Northrup King Co. Research Center, Stanton, MN, 55018.

Close to 5700 immature embryos were shot using a Bio-Rad Model 100 PDS-1000/He particle gun, using a set of basic culture conditions. A number of specific factors were compared, such day of post -shot selection initiation , and embryo plating method. In addition, several variables such as days between ear harvest and explanting, and male and female parent were tracked for all plates. Trends in the number of stable Transformants Per 100 Embryos (TPE) considering these variables is reported. All constructs were coshot with a PAT containing construct and selected on (phosphinothricin) containing medium. There was no significant difference between 0.25 M and 0.5 M mannitol in the gelrite solidified basal medium for a 4 hr pre/18 hr post shoot treatment. Both mannitol treatments are 5-7 times better than no osmotic treatment. Embryos placed on medium 1,2, or 3 days before shooting showed much higher TPE 's than those shot on day 0 or days 4-through 8. For all embryos shot, the TPE was 1.6. For Hi Type II genotypes, the TPE was 2.2 . For several specific crosses within Hi Type II , TPE's are compared. Shooting 1660 embryos of Hi Type II crossed with various elite materials yielded only 1 stable. Very little difference in TPE was seen for embryos from ears stored in the refrigerator for 0-14 days prior to explanting.

JS-8

DNA Transformation Using Electrically Charged Tungsten Microelectrodes. T. J. HARRINGTON and E. Aamodt. L. S. U. Medical Center, Department of Biochemistry, Shreveport, La. 71130

DNA mediated genetic transformation has opened the door to molecular engineering of numerous species. Unfortunately, many other species have so far resisted transformation often because DNA cannot be moved into their germ cells by current methods. An alternate approach was sought where DNA is electrophoresed onto the tip of a tungsten microelectrode and the electrode inserted into the cell nucleus to effect transformation. This research addresses two questions: Can DNA be electrophoresed onto the tip of tungsten microelectrodes and can tungsten microelectrodes carry DNA into the germ line cells of the nematode *C. elegans* to effect transformation? DNA could be electrophoresed onto the tips of tungsten microelectrodes and these electrodes could be used to transform the nematode *C. elegans*.

JS-9 Factors Enhancing *Agrobacterium*-Mediated Transformation of Peanut (*Arachis hypogaea* L.). **M. EGNIN, A. Mora and C. S. Prakash. School of Agriculture, Tuskegee University, Tuskegee, AL 36088**

Several factors were investigated to facilitate enhanced transfer of foreign genes into peanut using disarmed *Agrobacterium tumefaciens* with the screenable marker *gus*A gene: peanut genotype, explant type, bacterial strain, culture medium, the use of acetosyringone and treatment of explants prior to cocultivation. Hypocotyl and leaf explants were isolated from *in vitro* grown peanut seedlings (6-8 d) and cocultivated with bacterial culture for 5 days. The Valencia cv. New Mexico was more competent to transformation than Florunner, GA runner, Sunrunner or Southrunner. The *Agrobacterium* strain EHA 101 was superior in facilitating the transfer of marker genes to peanut cells when compared to the strain C58. Explants rinsed with 1/2-strength MS solution prior to cocultivation significantly increased the transformation. The addition of acetosyringone during bacterial culture at 50 μM or 200 μM resulted in the improved *gus*A expression in leaves but not in hypocotyls. The polarity of the hypocotyl was critical as upright orientation during cocultivation resulted in the improved transformation and shoot regeneration frequency. Several putative transgenic peanut shoots have been obtained and are being tested for gene integration and expression.

JS-10 Biotechnology Products from Animal Cells. A.S. LUBINIECKI. SmithKline Beecham Pharmaceuticals, King of Prussia, PA 19406

Recombinant DNA-derived proteins and monoclonal antibodies are prepared from continuous cell lines in increasing numbers since the first products were licensed in 1986. Several different types of bioreactor and cultivation technology can be successfully employed for this purpose. The inherent advantages and disadvantages of each should be matched to desired quantity and quality of protein as well as organizational resources. Examples of this principle will be shown. Another important feature of biotechnology products is their exceptional record with respect to process safety concerns. This feature reflects the use of characterized cell banks, well-designed processes capable of validation, and sophisticated analytical technology to characterize the essential molecular properties of the active ingredients. Future research and development efforts are likely to provide an even broader spectrum of vaccines, antibodies, therapeutic proteins, genetically modified cells and gene therapies for human use.

JS-11 Genetics and Molecular Biology of Gene Transfer, Amplification and Expression in Recombinant Chinese Hamster Ovary Cells

Florian M. WURM, Genentech Inc., South San Francisco, CA 94080, USA

I will address questions regarding the transfer and chromosomal integration of exogenous DNA in CHO cells and the amplification of the recombinant DNA. These methods result in stable high level expression of the transgene. I will elaborate on the heterogeneity of copy number, chromosomal location and structure of integrated sequences with respect to genetic stability and expression. In particular I will show slides from in-situ hybridizations showing primary intregration loci and of chromosomes which contain transgenic DNA at very high copy numbers. Cells with "amplified" sequences were obtained after exposure to methotrexate as a selective agent.

Also, I will discuss a new method for integration of exogenous DNA into regions of transcriptionally active regions of the CHO genome using homologous sequences derived from these cells. This approach, termed "Retrotargeting" enploys the use of fragments from deficient retroviral genomes which are present in the genome of CHO cells at high copy numbers.

JS-12 Genetic and Phenotypic Traits Observed During Development, Implementation and Monitoring of Large-Scale Mammalian Cell Culture Manufacturing Processes. S. ROBERT ADAMSON, Mammalian and Microbial Cell Sciences, Genetics Institute, Andover, MA 01810

Chinese Hamster Ovary (CHO) cells have been used extensively for the large-scale production of recombinant human proteins for therapeutic use. To express a protein at high levels, the cloned gene(s) is introduced into the CHO cell genome and amplified to high copy number using an appropriate, selectable, amplifiable marker. Suitable candidate cell lines are then adapted for growth in serum-free suspension culture and cells are evaluated with respect to various genotypic and phenotypic parameters prior to final cell line selection and cell banking. At Genetics Institute, cells are grown in a limited duration semi-continuous culture process. Key parameters are monitored throughout the process, both at the cellular level (DNA/RNA and product secretion) and at the product characterization level, to ensure that protein of appropriate quality is produced consistently throughout the production cycle. With experience accumulating from ten (10) plus years of manufacturing products using CHO cells, we are continually increasing our understanding of how intrinsic and extrinsic parameters effect protein structure. For example, the structure of carbohydrate moieties on recombinant glycoproteins has been reported to be susceptible to environmental conditions and duration of culture. A number of these experiences/observations will be described in case study format. The presentation will conclude with a description of the design and rationale for our current process and product monitoring approach in the context of general industry practice.

JS-13

Monitoring Monoclonal Antibody Production. W. G. ROBEY[1], J. Brackett[1], K. Cousineau[1], G. Gall[1], B. Peterson[1], A. Annapragada[1], H. Wang[2]. Rare Reagent Development, Abbott Laboratories, North Chicago, IL[1] 60064-4000, PerSeptive Biosystems, Cambridge, MA[2]

The metabolic processes that control efficient production of monoclonal antibodies by hybridoma cells grown in bioreactors are exceedingly complex and have been the subject of recent modeling studies. Modeling efforts have evaluated the effects of critical metabolic parameters (oxygen, glucose and glutamine consumption compared to ammonia and lactic acid production) on product formation. Concentrations of metabolites in bioreactor media were measured using an Abbott Vision® utilizing endpoint enzymatic assays. The monoclonal antibody produced was monitored using a PerSeptive Biosystems INTEGRAL™ Micro-Analytical Workstation operated in a simple capture and elution format. The capture antigen was biotinylated and immobilized on a PerSeptive BA (Strepavidin) ImmunoDetection™ (ID™) cartridge. Product antibody was captured in phosphate buffer, eluted with hydrochloric acid and quantitated by UV spectrophotometry. The limit of detection for the model antibody was 1 ug/mL and the assay dynamic range extended through 250 ug/mL. The combination of these instruments permitted rapid determinations of the effects of metabolite concentrations on product monoclonal antibody production in bioreactors maintained at different test conditions. Strategies for metabolic modeling of bioreactors in steady-state and perturbed conditions will be discussed.

V-1 Cell-Extracellular Matrix Interactions and Control of Tissue-Specific Transcription Factors. S. R. FARMER. Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118.

The process of differentiation during development involves the migration of proliferating stem cells to specific locations within the organism followed by growth arrest and induction of tissue-specific genes. During this process, cells encounter a dynamically changing microenvironment of extracellular matrix, soluble factors and different cell types which ultimately influences the morphology of the cell. These changes in cell morphology may have a direct role to play in regulating the expression of the differentiated phenotype. To understand the role of cell-extracellular matrix interactions in regulating the switch between growth and differentiation, we are studying the expression of the myoD and C/EBP families of transcription factors that control the differentiation of mesenchyme into muscle and fat, respectively. During the differentiation of myoblasts into multinucleated myotubes there is an elaborate change in cell morphology which involves the expression of contractile proteins. Induction of the myogenic transcription factor, myogenin, which regulates this process is dependent on adhesion of the myoblasts to an extracellular matrix. In contrast, differentiation of preadipocytes into adipocytes is accompanied by change in cell morphology to a more rounded shape and an extensive down-regulation of the cytoskeleton. Expression of the fat gene program is inhibited in preadipocytes cultured on fibronectin and enhanced in non-adherent cells. Studies are now in progress to determine the role of myogenin and C/EBPs in regulating the morphological differentiation of mesenchyme and to assess the importance of cell-extracellular matrix interactions in controlling this process.

V-2 No Abstract Submitted (H. Reddi)

V-3 Dynamic Reciprocity Revisited: A Continuous, Bidirectional Flow of Information Between Cells and the Extracellular Matrix Regulates Mammary Epithelial Cell Function. C.D. ROSKELLEY and M.J. Bissell. Laboratory for Cell Biology, Life Sciences Division, Lawrence Berkeley Lab., Berkeley CA, 94720.

Interactions between cells and the extracellular matrix (ECM) generate two classes of signals, mechanical and biochemical. In the case of the mammary epithelial cell both are required to initiate ECM-dependent expression of the abundant milk protein ß-casein. Mechanical signals induce a cellular rounding, while biochemical signals are associated with an increase in tyrosine phosphorylation. In a larger context, these individual transduction components are part of a complex signalling hierarchy that ultimately leads to the emergence of a fully-functional lactational phenotype. Furthermore, the assembly and disassembly of this hierarchy, which also occurs cyclically *in vivo*, are constantly modulated by dynamic and reciprocal interactions that take place within a functional unit composed of both the cell and the ECM.

V-4 Steroid Receptor Signaling Mechanisms. DEAN P. EDWARDS, Dept. Pathology, Univ. Colo. Health Sci. Ctr., 4200 E. 9th Ave., Denver, CO 80262

Steroid hormones and their cognate intracellular receptors represent a major signal transduction pathway by which extracellular molecules regulate gene expression. As members of the super family of ligand-dependent transcription activators, the signaling pathway for steroid receptors resides primarily in the nucleus and involves a sequence of molecular events that converts receptors from an inactive to an active state. As a model system our laboratory studies the human progesterone receptor (PR) in breast cancer cells. In the absence of hormone, PR associates with other cellular proteins to form an inactive oligomeric complex. Steroid binding induces a conformational change in the structure of PR which promotes its dissociation from the oligomeric complex, dimerization and binding to specific palindromic sequences of target genes. A nuclear accessory factor, identified as the chromatin high mobility group protein HMG-1, is required for high affinity interaction of PR with DNA. Binding to DNA in turn increases transcription by mechanisms that are not well defined. Several lines of evidence suggest that phosphorylation of PR also plays a role in modulating function including a rapid hormone-dependent hyperphosphorylation of PR that accompanies activation and that mutation of phosphorylation sites reduces receptor transcriptional activity. In addition to this "classic" steroid activation pathway, nuclear receptors have the capability to be activated in the absence of ligand by various membrane acting agents. This suggests a link between nuclear steroid receptors and other signal transduction pathways and the possibility to modulate nuclear receptors by multiple alternate pathways.

V-5 Coupling of Multiple Signal Transduction Pathways with Steroid Hormone Response Mechanisms: Implications for the Nuclear Receptor Family and Tissue Specificity of Hormone Response. STEVEN K. NORDEEN, Dept. Pathology, Univ. Colo. Health Sci. Ctr., Denver, CO 80262

Human progesterone receptor and rodent glucocorticoid receptor mediated induction of a mouse mammary tumor virus promoter is modulated by intervention with a variety of cellular signaling pathways. For example, activation of protein kinase A, protein kinase C, or a tyrosine kinase-dependent pathway with EGF potentiates the hormone response up to ten-fold in a cell type dependent fashion. Activators of protein kinase A can also elicit agonist activity in potent steroid antagonists, a finding with potentially important clinical ramifications. In contrast, the purines isobutylmethylxanthine (IBMX) and 2-aminopurine (2AP) will inhibit the hormone response by up to 90% in the mammary cells. The inhibitory effect of IBMX is rapidly imposed and stable whereas extended exposure to 2AP will reverse the inhibitory action and after 18-24 h begin to potentiate induction. In contrast to these inhibitory actions in mammary carcinoma cells, IBMX and 2AP strongly potentiate responses in fibroblasts. Thus, I propose that apparent tissue specificity of steroid response can arise via the differential coupling of steroid response mechanisms with cellular signal transduction pathways. This coupling does not alter glucocorticoid receptor phosphorylation and along with data on other members of the nuclear receptor family forces us to reconsider our conception of this family as receptors. A more global view of the role of this family envisions them instead as integrators of various signal transduction events in the cell.

V-6 Signal Transduction by the Receptor for Interleukin-3. STEVEN M. ANDERSON. University of Colorado Health Sciences Center, Deptartment of Pathology, 4200 E. 9th Ave., Denver, CO 80262

Stimulation of factor-dependent hematopoietic cells with cytokines such as IL-3 results in the rapid appearance of several phosphotyrosine-containing proteins, including the β subunit of the IL-3 receptor and the SH2-containing adapter protein shc. Investigations with a murine IL-3 dependent cell line indicate that multiple protein tyrosine kinases are activated following IL-3 stimulation including JAK2, *fyn*, *hck*, and *lyn*. Over-expression either of *fyn*, *hck* or *lyn* in 32D cl3 cells did not alter the sensitivity of the cells to IL-3, however, it altered the pattern of tyrosine-phosphorylation observed in the presence or absence of IL-3 stimulation. The *hck* tyrosine kinase is associated with the tyrosine-phosphorylated β subunit both *in vivo* and *in vitro* using bacterial fusion proteins containing specific regions of the *hck* protein. This suggests that that *hck* may have a specific function in signal transduction by the IL-3 receptor. This hypothesis is being explored with the use of dominant negative mutants of different tyrosine kinases to assess which kinase(s) are required for mitogenic signaling by the IL-3 receptor and which signal transduction pathways are activated by the various activated kinases.

V-7 Oncogene Signaling: Identification of Cell-Specific Factors Controlling Selective Gene Expression. A GUTIERREZ-HARTMANN, AP Bradford, KE Conrad, and B Wasylyk. University of Colorado HSC, Denver, CO 80262 & CNRS-LGME, Strasbourg, France.

The signaling pathway of the valine 12 Ras oncogene appears to utilize components that are common to many distinct cell types. Thus, the precise mechanisms by which oncogene signal tranduction pathways elicit cell-specific responses remain an important question in biology. To elucidate the molecular mechanism by which the Ras signaling pathway activates a cell type-specific gene, we have used the pituitary-specific rat prolactin (rPRL) promoter as a target of oncogenic Ras and Raf in GH4 rat pituitary cells. Using a transient co-transfection approach, we have shown that co-transfection of oncogenic V12Ras selectively stimulates the pituitary-specific rat prolactin (rPRL) promoter, and fails to activate the rGH or viral (RSV, SV40 or CMV) promoters. Additionally, we have identified and ordered the functional components of the Ras signaling cascade in GH4 pituitary cells, from the membrane to the nucleus, as: ras→raf kinase→MAP kinase→Ets-1/GHF-1. Furthermore, co-transfection of both the widely-expressed nuclear proto-oncogene transcription factor, Ets-1, and the pituitary-specific transcription factor, GHF-1/Pit-1, in a transfection-reconstitution assay in HeLa nonpituitary cells which is devoid of both of these factors, results in a synergistic enhancement of both basal and Ras-induced rPRL promoter activity. Also, using expression vectors encoding amino- and carboxy-terminal truncations of Ets-1, we have mapped the domain of Ets-1 required for the Ets-1/GHF-1 interaction to a region upstream of its DNA-binding domain. Finally, using 5' deletions and site-specific promoter mutants, we have mapped the Ras responsive element of the rPRL promoter to a composite element which contains both an Ets and an adjacent GHF-1 site. These data indicate that a functional interaction of these two factors confers a pituitary-specific response to the Ras signaling pathway, and thus provides critical molecular details by which the generic Ras signal is interpreted in a pituitary-specific manner by the GH4 nuclear transcription machinery.

V-8 **Telomerase activity is expressed in cycling but not in quiescent hematopoietic progenitors from adult human bone marrow** CHOY-PIK CHIU, Visia Dragowski, Nam Woo Kim, Terry E. Thomas, Peter M. Lansdorp and Calvin B. Harley. Geron Corporation, Menlo Park, CA 94025 and the Terry Fox Laboratory, Vancouver, British Columbia, Canada V5Z1L3

The loss of telomeric repeat sequences may serve as a mitotic clock which signals cell senescence and exit from cell cycle. Telomere shortening has been observed in normal human somatic cells upon ageing *in vivo* and *in vitro*. Telomerase, an enzyme which maintains telomere length by synthesizing telomeric repeats *de novo*, has so far been found only in cells with unlimited replicative potential such as the reproductive cells in testes, immortal cell lines and cancer tissues, but not in normal somatic cells. We have now examined telomerase expression in FACS sorted subpopulations of hematopoietic cells from adult human bone marrow using a sensitive PCR based telomerase repeat amplification protocol (TRAP). Telomerase activity was not found in the most primitive, quiescent hematopoietic cells with a $CD34^+CD71^{lo}CD45RA^{lo}$ phenotye. Interestingly, low levels of telomerase activity could be transiently detected when these cells were cultured in the presence of cytokines. Telomerase activity was also detected in the early progenitors ($CD34^+CD71^+$) but was down regulated when stimulated with cytokines. The more mature CD34- cells did not express any telomerase activity. Taken together, our results showed that telomerase activity is found in activated but not in quiescent hematopoietic progenitor cells and is down-regulated upon proliferation and differentiation. We have previously shown that telomere shortening can be observed in such hematopoietic cell cultures. The transient activation of telomerase activity described here is therefore unable to prevent telomere shortening in cultured hematopoietic cells. Alternatively, the telomerase activity may be only present in a small subpopulation and stablisation of telomere length is masked in the mass culture.

V-9 Endothelial Cell Differentiation, Angiogenesis, and the Inhibition of Tumor Growth with Aging. A. PASSANITI, R. Pili, and C. Yang. Laboratory of Biological Chemistry, National Institute on Aging, Baltimore, MD 21224

Although cancer incidence increases with age, the growth and progression of many solid tumors is slow in the elderly. Host factors may contribute to tumor growth inhibition by inhibiting neovascularization. In these studies, the role of aged host factors in tumor angiogenesis was investigated. Endothelial cell (EC) proliferation, differentiation, apoptosis in vitro and angiogenesis in aged mice were examined. Melanoma (B16) and carcinoma (EHS) cells grew more slowly in aged than adult or young mice. Histologically, EHS tumors growing in aged mice had altered extracellular matrix and fewer, but abnormally dilated, blood vessels. Independent in vivo measurements of angiogenesis showed that old mice had a 3 to 5-fold poorer vascular response to bFGF than young mice. Extracts of tumors growing in old mice inhibited the differentiation of EC and accelerated EC apoptosis. Loss of focal adhesion contacts and dephosphorylation of cellular protein tyrosine residues, in particular focal adhesion kinase, mitogen-activated protein kinase, and cyclin-dependent cdc-2 kinase were associated with induction of TRPM-2 and fragmentation of cellular DNA consistent with the activation of EC apoptosis. Inhibition of protein tyrosine phosphatases with sodium orthovanadate prevented EC tyrosine dephosphorylation, delayed the onset of apoptosis and restored angiogenesis in aged mice. In an in vitro model of growth arrest and differentiation using extracellular matrix extracts (Matrigel), bFGF or orthovanadate catalyzed EC differentiation by regulating the levels of p21 (waf1/cid1/sdi1) expression. Unregulated expression of p21 resulted in EC apoptosis. Therefore, cellular protein phosphatases are involved in EC apoptosis and prevent differentiation. Inhibition of these enzymes may enhance angiogenesis in vivo. These models will be useful in further characterizing the role of aged host factors in angiogenesis and tumor growth.

V-10 Transcription Events in Cellular Senescence

(J. Campisi) No Abstract Submitted

V-11 Molecular Genetic Studies of Human Cellular Senescence. P. J. HENSLER and O. M. Pereira-Smith. Roy M. and Phyllis Gough Huffington Center on Aging, Baylor College of Medicine, Houston Texas 77030

Normal cells in culture exhibit limited division potential which has been used as a model for cellular senescence. In contrast, tumor derived, carcinogen or virus transformed cells are capable of indefinite division (immortal). Previous fusion experiments of normal human diploid fibroblasts with immortal human cells yielded hybrids having limited life span, indicating that cellular senescence was dominant and immortality due to recessive changes. Fusions of various immortal human cell lines with each other led to the identification of four complementation groups for indefinite division. To identify chromosomes and ultimately the genes involved in growth regulation, microcell fusion experiments were performed to introduce single human chromosomes into cell lines representing each of the four complementation groups. Introduction of a normal human chromosome 4 induced senescence only in immortal cell lines assigning to group B. During this analysis, a fragment of chromosome 4 was generated which contains approximately 800kb of DNA which can induce senescence. In addition, introduction of human chromosome 1 into immortal cell lines assigned to each complementation group demonstrated that this chromosome could specifically induce senescence in the immortal human cell lines assigned to group C. Using positional mapping of microcell hybrids which have escaped from senescence and become immortal, two putative senescence loci have been identified on the q arm of human chromosome 1. Several approaches are currently being pursued in an attempt to identify the growth regulatory genes on chromosomes 4 and 1.

V-12 Low Shear Stress Of NASA Rotating Wall Vessel (RWV) Increases CO_2 And Acid Production While Supporting Differentiation in Three-Dimensional Cultures. J.M. JESSUP, A. Nachman, and R.D. Ford. Laboratory of Cancer Biology, Deaconess Hospital, Boston, MA 02115.

The NASA-designed RWV simulates a 0.2 μg field by rotating cells on microcarrier beads around the vertical axis in a fluid-filled chamber with an initial shear stress of only 0.3 dynes/cm^2. Since RWV cultures of the human colorectal carcinoma MIP-101 produce three-dimensional cell masses which are more differentiated than monolayer cultures, our purpose was to determine whether differentiation in the RWV was associated with the production of more acid metabolites than when cells were cultured in monolayer cultures in T-25 flasks where differentiation does not occur. MIP-101 cells were cultured in RPMI 1640 with 10% FCS on Cytodex 3 microcarrier beads (15 - 50 cells/bead and 5 mg beads/mL) in 50 mL RWVs or as monolayers in medium-filled 70 mL T-25 flasks. Cell counts, glucose, pH, PCO_2, and PO_2 were measured on alternate days for up to 8 days. Differentiation was assessed by immunohistochemical detection of the tumor marker carcinoembryonic antigen (CEA). Proliferation and consumption of glucose and oxygen were similar. However, production of both H+ and CO_2 and expression of CEA was greater in the RWV than in monolayer T-25 cultures. These results suggest that low shear stress is associated with increased production of acid metabolites without significant changes in either glucose or oxygen consumption. The expression of CEA may be related to the metabolic changes caused by this low shear stress.

(Supported by grant NAG 9-650 from NASA)

V-13 Microgravity-Suppressed Peripheral Blood Mononuclear Cell (PBMC) Locomotion Is Restored By Iron-Transferrin Supplementation. R.P. PIZZINI and N.R. Pellis. KRUG Life Sciences and NASA Johnson Space Center, Houston, TX 77058.

Normal immune function requires the locomotion of peripheral blood mononuclear cells (PBMC) through the extracellular matrix. This ability is impaired in microgravity. Iron-transferrin (Tf) is required for PBMC locomotion and enhances locomotion in the presence of breast tumor cells. Therefore, we studied the effect of Tf supplementation on microgravity-suppressed locomotion. Normal human PBMCs ($1x10^6$/ml) were placed in flasks (STATIC) or slow turning lateral vessel (STLV) and high aspect rotating vessel (HARV) culture systems that approximate microgravity. Cell locomotion was evaluated on days 0-3. PBMCs ($2.5x10^5$) were placed on polymerized type I collagen in 8-chamber slides and overlayered with 100 µl media (control) or media supplemented with 20 µg/ml iron-poor (apo-Tf) or iron-rich (holo-Tf) transferrin. On day 0, holo-Tf stimulated locomotion (media 464.37±5.31µm, apo-Tf 463.28±16.80, holo-Tf 515±8.37; $p<0.05$ vs. media & apo-Tf). Locomotion was suppressed in STLV and HARV on days 1-3. Holo-Tf provided restoration of locomotion to STLV and partial restoration in the HARV as compared with controls.

Day 3	*Media*	*apo-Tf*	*holo-Tf*
STATIC	590.1±8.3	587.3±11.9	615.2±6.2
STLV	536.8±8.3•	495.0±18.9*•	628.0±7.3**
HARV	428.4±9.5••	469.2±23.6•	510.5±9.7*••

$p<0.05$ *vs media, **vs media & apo-Tf
•vs STATIC, ••vs STATIC & STLV

In vitro iron-Tf supplementation may provide additional insight into the mechanisms of microgravity-induced impairment of cellular locomotion as well as potential methods to circumvent these effects in space. This research was funded by the Gillson-Longenbaugh Foundation and NASA Grant #NAG 9-664.

V-14 Three Dimensional Multicellular Systems in vitro: NASA Bioreactor and other techniques. LEONID MARGOLIS, Wendy Fitzgerald, Neomi Amichai, Boris Baibakov, Svetlana Glushakova, and Joshua Zimmerberg. Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, NIH, Bethesda MD 20892.

We used NASA Bioreactor as well as other techniques to culture cells in three-dimensional structures. NASA Bioreactor provided an efficient way for culturing mouse melanoma cell. Some of these variants fail to grow in other rotating vessels due to their defective adhesion. The effects of the way of culturing of human lymphocytes and mouse kidney cells on cell propagation and differentiation will be discussed. Culturing of blocks of various tissues including mouse kidney and thymus, human tonsils and adenoids, using various techniques showed that histocultures (culturing on top of floating collagen gel-sponges) have some advantages over other methods of tissue culture. In NASA Bioreactor lymph tissues become lymphocyte-depleted, while stromal elements remain viable for a long period and can be repopulated with fresh lymphocytes. This provides a new in vitro system for studying the re-population (homing) phenomenon. The result on re-population of lymph tissue with lymphocytes in NASA Bioreactor will be reported.

V-15 Simulated Microgravity Enhances Extracellular Matrix Protein Expression in Cultured PC12 Pheochromocytoma Cells. J. LIU, D.L. Galvan*, B.R. Unsworth* and P.I. Lelkes, Univ. Wisc. Med. School and Dept. of Biology, Marquette Univ., Milwaukee, WI 53233.

Previous studies have shown that simulated microgravity (SM) in NASA's rotating wall vessels (RWVs) generates favorable conditions for the induction of tissue-specific differentiation of cultured cells *in vitro*. Using rat PC12 pheochromocytoma cells as a model system, we are studying the effects of SM on neuroendocrine differentiation in the adrenal medulla. PC12 cells, seeded onto different microcarrier-beads, (Cytodex3 and CultispherGL) were grown for 20 days in STLV-type RWVs, in conventional stirred fermentors and under static 2-D condi-tions. Upon completion of the experiments RNA was ex-tracted from the ensuing cellular aggregates (up to 0.75 cm diameter for cells grown on Cultisphere in the STLV). The expression of extracellular matrix (ECM) protein genes was analyzed by semi-quantitative RT-PCR and compared to that of constitutively expressed house-keeping genes, such as GAPDH. PC12 cells expressed fibronectin under all conditions studies, however the level of fibronectin expression was significantly enhanced in SM, in particular when the cells were cultured on Cultisphere beads. Moreover, SM slightly induced the expression of collagen IVα1, which is strongly expressed in the intact adrenal, but is absent in conventionally cultured PC12 cells. Our results suggest that SM is a valuable culture modality for inducing tissue-specific gene expression, and that under SM PC12 cells seem to assume a more histiotypic epitheliod phenotype . (Supported by a grant from NASA)

V-16 Human Renal Epithelial Cells in Culture Differentiate under simulated Microgravity. T.G. HAMMOND, D.L.Galvin, T.J. Goodwin, and P.I.Lelkes. Milwaukee Clinical and Madison Campuses, University of Wisconsin, WI, 53792, and NASA/Johnson Space Center, Houston, TX 77058.

Scavenger pathway receptors on the luminal surface of renal proximal tubular cells are thought to mediate many common forms of pharmacological injury. Mechanistic studies on renal toxicity are severely limited by dedifferentiation of proximal tubular cells in culture, including loss of expression of scavenger pathway receptors. Further, normal human renal cells have been difficult to obtain, cumbersome to purify, and impossible to maintain in a differentiated form in culture. We report harvest of human renal epithelial cells, and isolation of highly purified cell populations. These cells lose their polarity and differentiated features in conventional 2-D and suspension culture. Under simulated microgravity in NASA slow turning lateral wall vessels the cells maintain polarity. Two lines of evidence support the conclusion that these cells maintain proximal tubular differentiation in culture: 1. the cells are >99% positive for proximal tubular enzyme markers on flow cytometry analysis, 2. electron microscopy shows polarized features including a brush border, and endosomal differentiation including dense apical tubules. Human renal epithelial cells cultured under simulated microgravity provide a source of polarized mammalian cells for study. Microgravity culture will provide an unprecedented renal toxicity model as scavenger pathway receptor expression is optimized.

V-17 Microgravity Enhances Tissue-Specific Neuroendocrine Differentiation in Cocoultures of Rat Adrenal Medullary Parenchymal and Endothelial Cells. D.L. GALVAN(*), B.R. Unsworth(*), T.J. Goodwin(#), J. Liu(+) and P.I. Lelkes(+), (*) Marquette Univ., (+) Univ. Wisc. Med. School, Milwaukee, WI 53233, (#) NASA-JSC, Houston, TX 77058.

We previously reported that rat adrenal medullary PC12 pheochromocytoma cells differentiated towards the neuro-endocrine phenotype when cocultured with endothelial (RAME) cells derived from the same tissue. In extending our studies we capitalized on the enhanced cellular interactions observed under simulated microgravity (SM) in NASA's rotating wall vessels (RWVs). Cocultures of PC12 and RAME cells on microcarrier beads were maintained for up to 30 days either in a STLV-type RWV or, synchronously, in standard 2-D tissue culture flasks and in stirred fermentors. As expected, SM facilitated the organization of the cells into large (up to 7.5 mm diameter), histiotypic nest-like assemblies. In comparison to the conventional culture modalities, SM significantly enhanced the expression of neuroendocrine markers in PC12 cells, such as chromogranin A, PNMT, TH, DβH, and neurofilament, as assessed by immunocytochemistry. Similarly, SM inceased the gene expression of several catecholamine synthesizing enzymes, as inferred from quantitative RT-PCR. Specifically, tyrosine hydroxylase, dopa decarboxylase, and PNMT were significantly increased. Also, in several instances the incipient formation of capillary structures was observed, indicative of the bidirectionality of the differetiative interactions uner SM conditions. Our data suggest that the RWV system is suitable to evaluate the role of SM in promoting differentiative, organ-specific interactions in heterotypic co-cultures. (Supported by a grant from NASA)

V-18 Brief Exposure to Simulated Microgravity Affects Tyrosine Phosphorylation in PC12 Pheochromocytoma Cells. D.L. GALVAN*, B.R. Unsworth* and P.I. Lelkes, Univ. Wisc. Med. School and Dept. of Biology, Marquette University, Milwaukee, WI 53233.

We previously reported that the expression and specific activity of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis was decreased in adrenals of rats exposed to space flight. We hypothesized that this effect might have been caused by microgravity-induced alterations in cellular signalling pathways, in particular changes in protein phosphorylation. To test our hypothesis we seeded rat adrenal medullary PC12 pheochromocytoma cells onto microcarrier beads and briefly exposed the cultures for 30 minutes to simulated microgravity (SM) in a STLV-type rotating wall vessel (RWV). In parallel, synchronous cultures were exposed to shear forces in a conventional stirred fermentor and/or maintained under static conditions in a Petri dish. Upon termination of the experiments, the cells were lysed and the cellular proteins analyzed by SDS-PAGE and Western blotting using a polyclonal rabbit anti-phosphotyrosine antibody. Microgravity, but not any of the controls, was found to selectively tyrosine phosphorylate a unique protein band with a molecular mass of approx. 210 kD. Concomitantly, another protein band of approx. 186 kD was dephosphorylated in SM, but not in the control conditions. Our data support the notion that intracellular signalling can be affected by microgravity as provided in the RWVs. Current experiments are under way to assess details of microgravity-sensitive signal transduction pathways in PC12 cells. (Supported by a grant from NASA)

V-19 Increased Cytotoxicity of Bleomycin when used in Conjunction with Electroporation. R. HELLER[1,2], M. Jaroszeski[1], R. Perrott[1], J. Becker[3], H. Arango[3], P. Satyaswaroop[4] and R. Gilbert[2]. University of South Florida, Depts. [1]Surgery, [2]Chem. Engineering, [3]Ob/Gyn Tampa, FL 33612. Hershey Med. Center, Dept. [4]Ob/Gyn Hershey, PA 17033.

The administration of electrical pulses to living cells temporarily induces formation of pore-like structures (electroporation) in the membrane which gives molecules intracellular access. Recently, electroporation (EP) has been used to facilitate the entry of chemotherapeutic agents into cells. This study was conducted to examine the influence of EP on the cytotoxic potential of bleomycin on various tumor cell types. These included B16 murine melanoma, Ishikawa human endometrial cancer, LN1 human ovarian cancer, and T47D human breast cancer. Each cell line was exposed to four concentrations of bleomycin ranging from 10^{-3} to 10^{-10} M in the presence and absence of electrical treatment. EP consisted of eight 100 μs rectangular DC pulses with an electric field strength of 1500 V/cm. After treatment, the cells were plated for 24 hours. Then, a thiazoyl blue survival assay was conducted. For all tumor cells treatment with EP decreased the IC_{50} by at least two orders of magnitude. The following decreases in IC_{50} were observed: 300 fold for B16 cells; 3,000 fold for Ishikawa cells, 125 fold for LN1 cells; and 700 fold for T47D cell lines. Future studies will assess the effectiveness of this technique using additional antitumor agents for these tumor cells and for cells from other types of solid tumors. (This work supported by U.S.F. Depts. of Surg. and Chem. Engineering and Genetronics, Inc., San Diego, CA.

V-20 No Abstract Submitted (Kerbel)

V-21 Neoplastic Transformation of Human Fibroblasts in Culture--A Multi-stepped Process
J. J. MCCORMICK and V. M. Maher, Carcinogenesis Laboratory -- The Cancer Center, Michigan State University, East Lansing, MI, 48824, USA
The transformation of human fibroblasts *in vitro* to malignant cells has never been observed to occur spontaneously or as a result of carcinogen/mutagen treatment. Because multiple, mutational-type events are no doubt required for malignant transformation, a necessary early event in the process *in vitro* is the generation of a cell strain with a greatly extended or infinite life span. By transfection of a v-*myc* gene into human fibroblasts, selection of a clonal population expressing the v-*myc* protein, and some additional rare chance event(s), we have been able to generate a diploid, infinite life span cell strain (MSU-1.0). A spontaneous growth factor variant cell strain arose in the MSU-1.0 population named MSU-1.1. The MSU-1.1 cells can be transformed to malignancy by transfection of the various *ras* oncogenes, as well as the *fes* oncogene. The *sis* oncogene (a PDGF-B homolog) transforms the cells so they form benign tumors (fibromas). The MSU-1.1 cells can also be transformed to malignancy by treatment with benzo(a)pyrene diol epoxide, various other chemical carcinogens, or ionizing radiation. Cell fusion data indicate that the activity of different suppressor genes is lost in the immortalization step and in the derivation of MSU-1.1 cells from MSU-1.0 cells. These data, along with information on which oncogenes are activated, indicate that 6-7 independent genetic changes are required for the cells to become malignant. (Research supported DHHS Grant AG11026 and CA60907 and DOE Grant ER60524.)

V-22 **Defects in Cell Cycle Control and Differentiation in Multistage Cancer of Mouse Epidermal Cells.** M. KULESZ-MARTIN. Roswell Park Cancer Institute, Buffalo, NY 14263
Cancers in mouse epidermis, like human epithelial cell cancers, require the accumulation of multiple changes in gene expression. Stages defined by histopathology are normal, preneoplastic (including hyperplastic, benign tumor, and dysplastic) and neoplastic (including focal invasion of underlying tissue and squamous cell carcinoma). Squamous cell carcinoma may progress from well differentiated to moderately differentiated, anaplastic and spindle cell types. Carcinomas metastasize at a frequency which may be dependent upon initiation or promotion agents and protocols. Model systems using mouse epidermal keratinocytes have permitted quantitation of initiation and tumor promotion in vitro based on the frequency and size of colonies growing under conditions which favor normal cell differentiation. Evaluation of benign tumor formation or well differentiated malignant tumors requires transplantation in vivo by grafting at the site of origin. Cultures of epidermal cells at discreet stages of carcinogenesis, including a cell lineage developed from an epidermal cell clone treated with 7,12-dimethyl-benz[α]anthracene, have provided targets for evaluation of the activity of oncogenes introduced at discreet stages of transformation and a source of cells to compare stage-specific expression of oncogenes and tumor suppressor genes. In vivo and in vitro studies have determined that epidermal carcinogenesis is associated with changes in keratin genes, *Ha-ras* and *c-fos* protooncogenes, protein kinase C, TGF-ß and TGF-ß receptors, integrins and the tumor suppressor gene *p53*. The functional contribution of each to cancer development, the stage-specificity of accumulated defects and whether the pattern of their expression in a given tumor is initiator- or promoter- dependent are remaining questions to be approached in epithelial model systems.

V-23 Induction, Progression, and Prevention of Carcinogenesis in Cultured Respiratory Epithelial Cells. V.E. STEELE. Division of Cancer Prevention and Control, National Cancer Institute, NIH, Bethesda, MD 20892.

Respiratory epithelium is the major site of cancer mortality in both men and women is the U.S. Tissue culture methods have been used by a many investigators to study this process for at least two decades. Malignant transformation can be induced in rat tracheal epithelial cells by a wide variety of direct and indirect acting carcinogens, including polycyclic hydrocarbons, nitrosamines, metal salts, and cigarette smoke condensate. Tumor promoters enhance the transformation frequency, but do not by themselves transform cells. The cancer process evolves through multiple stages, many of which are discernable in vitro. The earliest indication of neoplastic progression is a morphological transformation characterized by colonies of what would be called dysplastic cells if seen in a histological section. These cells are characterized by increased nuclear size, altered nuclear shape and density, increased mitosis, aberrant differentiation and senescence. Three or more distinct morphological classes can be identified. Clonal evolution leads to the appearance of increasingly aneuploid and anchorage independent cells. Eventually these cells form carcinomas when injected into nude mice. Recently this tracheal epithelial transformation system is proving to be very useful in identifying cancer preventing chemicals.

T-1 An *In Vitro* Approach to the Study of Hepatotoxic Agents with a Primary Culture System of Rat Liver Cells. D. ACOSTA, JR. College of Pharmacy, University of Texas, Austin, TX 78712.

Exposure to drugs and chemicals often results in toxicity to living organisms. We have come to appreciate the fact that not all compounds are equally toxic to all parts of the living system, because the toxic actions of many compounds are manifested in specific organs. These organs are known as target organs of toxicity. This concept has developed into the evaluation of toxicants via their target organ specificity. The application of *in vitro* model systems to evaluate the toxicity of xenobiotics has significantly enhanced our understanding of drug- and chemical-induced target organ toxicity. *In vitro* models commonly used in target organ toxicity testing include: perfused organ preparations, isolated tissue slices, single cell suspensions and cell culture systems. The expression of target organ toxicity ranges from subtle abnormalities of cellular organelles to permanent loss of organ function. Because each organ system is characterized by a large degree of structural and functional heterogeneity, the assessment of toxicity *in vivo* is often complicated. In this regard, *in vitro* model systems can be used to define general mechanisms of toxicity or to screen potentially toxic drugs and other chemicals. An essential consideration in attempting to validate *in vitro* systems is that the data obtained *in vitro* should always be correlated to observations made *in vivo*. A major goal of our laboratory has been the development of primary culture systems that retain differentiated functions and responses characteristic of intact tissues *in vivo*. Specifically, we have developed cellular models of primary cultures of rat liver cells to explore the mechanisms by which drugs or chemicals may be toxic to a key organ of the body and to develop new techniques by which xenobiotics may be evaluated or identified as potential toxicants to living systems. The objective of this talk is to provide a brief overview of our approach to the study of target organ liver toxicology with *in vitro* cellular systems.

T-2 No Abstract Submitted (C. Ruegg)

T-3 Cultured Human Hepatocytes as *In Vitro* Models for Examining Drug Toxicity and Metabolism. R. ULRICH, Investigative Toxicology, Upjohn Laboratories, Kalamazoo MI 49007.

Cultured hepatocyte techniques have facilitated investigations on the consequences of xenobiotic exposure, including hepatic metabolism and target organ-specific toxicity. Our efforts have focussed on developing reliable methods for isolation, culture, and cryopreservation of human hepatocytes from organ donor tissues and the application of these cells in drug-cell interaction studies. Human hepatocytes, freshly isolated or cryopreserved, have applications in all phases of drug development (preclinical through clinical) for examining drug uptake and metabolism, and investigating mechanisms of hepatic toxicity. In the preclinical development phase, examining drug-cell interactions with cultured hepatocytes from the preclinical animal species (rat, dog, rabbit and monkey) and comparison with *in vivo* data provides a degree of precision to predictions made from studies involving human cells. Endpoints for *in vitro* studies cover a broad spectrum of cellular responses, and are based on relevant *in vivo* observations. They include toxicity markers, metabolic profiles, changes in organellar structure and activity, changes in cellular physiology, effects on signal transduction and gene expression. When untoward hepatic effects are observed in the clinical development phase, human hepatocytes can be used to help elucidate mechanisms and provide guidance back to chemistry analogue efforts. In these regards, *in vitro* studies with human hepatocytes have significantly influenced and enhanced the discovery and development of compounds from a diversity of structural and pharmacological classes, including antibiotics, anxiolytics, antidiabetics and lipid-lowering agents.

T-4 Cell and Tissue Systems *In Vitro*: The Next Best Thing to Being *In Vivo*? KENNETH L. AUDUS, Department of Pharmaceutical Chemistry, The University of Kansas, 3006 Malott Hall, Lawrence, Kansas 66045-2504.

The continuing development of a variety of *in vitro* cell and tissue systems permits the examination of cellular and molecular level mechanisms of toxicology and transport at specific endothelial and epithelial interfaces. An important and recent application of these *in vitro* systems has been in facilitating the rapid characterization of the so-called pharmaceutical properties (i.e., toxicity, metabolism, and transport processes) of new drug candidates. To validate the use of *in vitro* systems, both academic and industrial labs are currently attempting to correlate observations generated *in vitro* with studies run in parallel with *in vivo* models. While not always realistic, some of the *in vitro - in vivo* relationships have potentially important implications for a qualitative understanding of the toxicological and transport processes in specific tissue systems. In addition, these studies have provided a subtle reminder of the complex contributions of cell-cell interactions, hormones, environmental input, etc., to the toxicological and transport processes of a given tissue. As a consequence, researchers should also be able to consider the introduction of certain refinements into *in vitro* cell and tissue systems that make them more applicable to the study of cell and molecular biology as well as the pharmaceutical properties of new drug candidates. The purpose of discussion here is to outline the role of *in vitro - in vivo* correlations in basic biology studies and to introduce subsequent discussions of how *in vitro - in vivo* correlations have been successfully developed for understanding toxicological or transport processes of specific tissues.

T-5 P450 Induction and Bile-Acid Transport in Cultures of Sandwiched Rat Hepatocytes. E.L. LeCLUYSE, P.L. Bullock and A. Parkinson. Pharmacology, Toxicology, and Therapeutics, Center for Environmental and Occupational Health, University of Kansas Medical Center, Kansas City, KS 66160.

Historically, long-term cultures of rat hepatocytes have not been a widely used *in vitro* model system in toxicology and pharmacology. This has largely been due to the lack of a satisfactory culture environment in which cell viability and liver-specific properties such as P450 enzyme activity and bile acid transport are maintained for prolonged periods. More recently, novel culture techniques have been developed whereby hepatocytes are "sandwiched" between two layers of extracellular matrix. Under these conditions, prolonged viability and improved morphological properties are observed including the formation of functional bile canalicular networks. Similarly, cytochrome P450 induction and bile acid transport are dramatically influenced by extracellular matrix configuration as well as media composition. In conclusion, rat hepatocytes can be cultured under conditions that re-establish near normal morphology and liver-specific gene expression, making long-term cultures of rat hepatocytes a useful tool for toxicological and biochemical studies.

T-6 *In Vitro-In Vivo* Blood-Brain Barrier Permeability Correlations of Receptor Selective, Opioid Peptides. THOMAS P. DAVIS, Victor J. Hruby, and Thomas J. Abbruscato, Department of Pharmacology, University of Arizona, College of Medicine, Tucson, Arizona, 85724

Confluent monolayers of primary and continuous passaged cultures of bovine brain microvessel endothelial cells (BMEC) have been suggested to model the blood-brain barrier (BBB). Increased lipophilicity has been previously suggested to increase BBB penetration. The intent of our study was to examine the effect of structural modification of the met-enkephalin analog DPDPE on lipophilicity and passage across the BMEC and to correlate these results to *in vivo* BBB permeability of the same opioid peptides. The BMEC consisted of a monolayer of confluent primary cells grown on polycarbonate (10μm) filters. Permeability coefficients were calculated on the basis of the diffusion of peptides across the BMEC. Lipophilicity of the peptides was determined using reversed-phase HPLC and calculating capacity factor (*k*). *In vivo* BBB permeability was determined after 30,60,120 minutes of perfusion by *i.p* or *i.v.* administration. BMEC permeability coefficients ranged from 4.55 to 92.00 cm/min (x 10^{-4}). Analysis of the regression line of permeability coefficients plotted against capacity factors and *in vivo* distribution to the brain yielded a correlation coefficient of 0.745 ($P < .01$) for capacity factors and 0.90 for *in vivo* data. The data provided in this study offer strong evidence that increasing peptide lipophilicity also enhances passage across the BMEC. Comparison of the permeability coefficients obtained from the BMEC system with those obtained from *in vivo* BBB studies suggest that the BMEC system may be very useful in predicting peptide (analog) passage across the *in vivo* BBB. (Supported by N.I.D.A. grant #DA06284)

T-7 Drug Transport Studies in the Intestinal Epithelial Cell Line CaCo-2: Correlation with Absorption in Vivo. C.A. BAILEY, Pharmaceutical Research and Development, Hoffmann-La Roche Inc., 340 Kingsland St., Nutley, NJ 07110

The CaCo-2 cell line was originally isolated from a human primary colonic adenocarcinoma. Under carefully defined and reproducible culture conditions, these cells can be induced to develop morphologic and biochemical characteristics of differentiated human intestinal epithelial cells. A model that employs these cells cultured on filters and maintained in transport wells readily lends itself to in vitro studies of drug transport and delivery. This transport model allows for the isolation of the epithelial absorptive function from other major factors that impact on overall oral bioavailability, such as first-pass metabolism and clearance. Studies of transport of drug molecules in this model can provide calculated apparent permeability coefficients, which can be compared with human intestinal permeability and overall bioavailability. In addition, information on the mechanism(s) of transport across the barrier can be obtained. Thus it can be determined whether the molecule is crossing via paracellular or transcellular routes, or is using an endogenous, active carrier system. Further correlations can be obtained between transport and molecular properties of the drug, allowing for predictive information feedback to the drug development process. These studies indicate that valuable information on intestinal drug absorption and in vitro/in vivo correlations can be obtained from the model.

T-8 New insights into *Interspecies Selectivity* using cell culture models of pesticide neurotoxicity. Belinda VERONESI, U.S. Environmental Protection Agency, HERL, NTD, RTP, NC.

Pesticides outrank all classes of neurotoxicants in terms of economic importance and environmental risk. Organophosphorous (OP) insecticides are of special concern due to their widespread use. The major biochemical targets of OPs are acetylcholinesterase and neurotoxic esterase, enzymes whose inhibitions can produce lethality or paralysis, respectively.

One of the most perplexing features of organophosphorous (OP) insecticide neurotoxicity is the highly variable biochemical, neuropathological and functional response recorded among animal species or even strains of the same species that have been exposed to OPs. This phenomenon, known as *interspecies selectivity,* has handicapped rigorous mechanistic investigations into pesticide neurotoxicity. A history of this phenomenon, conventional explanations for it and current in vitro approaches to elucidate it, will be discussed. The experiments presented will demonstrate that even in culture, cells derived from humans and rodent species, respond cytotoxically and neurotoxically differently to OPs. These data suggest that interspecies selectivity to OPs might be explained in terms of species specific cellular metabolism and target enzyme activities.

T-9 Technical Aspects of Using Primary Cultures of Nervous Tissue to Investigate Chemical Neurotoxicity. H.D. DURHAM, McGill University, Montreal, Canada H3A 2B4.
When assessing the neurotoxicity of an unknown, primary cultures of nervous tissue offer advantages over cell lines that may not express key properties that contribute to the selective vulnerability of specific neuronal types to the agent. The following issues will be discussed in relation to the use of primary cultures of nervous tissue. Solubility and Volatility of Test Chemicals: Many neurotoxicants are hydrophilic and solvents such as DMSO or ethanol must be used to solubilize such agents in culture medium. 0.05% DMSO is not cytotoxic when exposure is limited to a few days, but aberrant neurofilament phosphorylation and focal swelling of neurites may occur with exposures longer than one wk. If vaporization of both solubilizing vehicles and test compounds at 37°C is to be avoided, experiments must be conducted in sealed culture dishes. Metabolism: Many neurotoxicants are metabolites of protoxicants. Metabolites may be generated by coculture with hepatocytes or addition of S9 liver fraction. In either case, physical contact with primary neurons must be avoided. Evaluation of neurotoxicity: must take into account the multiple cell types that coexist in the culture and their relatively low numbers. In addition to general cytotoxicity and morphology, a number of neuron-specific properties may be measured including ion channel function and expression of neuron-specific proteins. Introduction of foreign DNA: Liposome-mediated transfection is not generally a suitable method to introduce foreign DNA into primary neurons; however, replication-defective viruses (eg. adenovirus) are highly efficient vectors for gene transfer into neuronal and non-neuronal cells.

T-10 Patch Clamp Technology in Neurotoxicological Evaluation. CHRISTOPH, Greg. DuPont Company, Central Research, Haskell Laboratory, Elkton Road, Newark, DE 19714.

Specifying the mechanism(s) of mammalian toxicity is highly relevant to the risk assessment process for pesticide and pharmaceutical products. The mechanistic data can also feed directly into the product discovery strategy, which typically involves numerous candidate compounds, so that unwanted effects can be systematically eliminated early in the selection process. Patch clamp methods refer to an electrophysiological analysis that provides precise information about the transmembrane flux of ions, and it is the tool of choice for initial investigation of chemicals that disrupt neuronal electrical activity. Neuroblastoma cells maintained in culture exhibit the same types voltage-dependent ionic currents as real neurons and are easily studied with tight-seal, whole-cell recording versions of the patch clamp method. Use of primary cultures of rat dorsal root ganglion neurons, although more tedious to prepare and maintain than neuroblastoma cells, permits more straightforward generalization to neurons *in vivo*. Examples of patch clamp studies with both types of cell preparations and analysis of voltage-dependent sodium and potassium currents will illustrate the utility of the methods in the context of pharmaceutical and insecticide development objectives.

T-11 *In vitro* Screens for Esterase-Inhibiting Neurotoxicants. M. EHRICH, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA 24061.

Organophosphates (OPs) are widely used in insecticides, lubricants, and plastics. Acute and delayed toxicity to man and animals can be predicted by capability to inhibit acetylcholinesterase (AChE) or neuropathy target esterase (NTE, neurotoxic esterase), respectively. These esterases are found in several cell lines, and our experiments have demonstrated that human neuroblastoma SH-SY5Y and mouse neuroblastoma NB41A3 can be used to distinguish neuropathy-causing OPs from among a series of direct esterase inhibitors. Although AChE and NTE inhibitions can be directly related to clinical manifestations and toxic outcome in animals exposed to OPs, this relationship is less clear with neuroblastoma cell lines. With neuroblastoma cell lines, OP-induced AChE and NTE inhibitions did occur, as they do in animals, at concentrations less than those causing loss of viability. Esterase inhibition, especially AChE inhibition, was not, however, a necessary prerequisite for lethality, as it is in animals demonstrating acute toxicity resulting from OP exposure. These results support the suggestion that robust *in vitro* screening methods for neurotoxicants should include consideration of specific targets and/or molecular mechanisms contributing to toxic manifestations *in vivo*, as cytotoxicity data alone could be misleading. (Supported by US EPA, HERL).

T-12 Xenobiotic-mediated Induction of Cytochrome P450 Gene Expression in Primary and Transformed Rat Hepatocyte Cultures: Differences in Expression and Induction Potential as Ascertained by RT-PCR. J.S. SIDHU, F.M. Farin, and C.J. Omiecinski. Dept. of Environmental Health, XD-41, University of Washington, Seattle WA 98195

A primary rat hepatocyte culture system has been established that accurately reproduces both liver-selective and xenobiotic-responsive gene expression patterns observed *in vivo*. Standard mRNA hybridization, protein immunochemical, and enzymatic assays are typically of sufficient sensitivity to detect these expression profiles in the primary cell cultures. We also have surveyed a number of hepatoma cell lines, including the rat H4IIE, FAO, C2Rev7, and human HepG2, for comparative P450 responsiveness. In contrast to the primary cells, all cell lines examined were refractory to phenobarbital induction, a hallmark feature of differeniated hepatocytes. This was depite detection of several other constitutive P450s in the cell lines and induction responsiveness to other prototypic inducers, such as the polyaromatic hydrocarbons. To assess the cell lines with increased sensitivity, reverse transcriptase-coupled PCR and confocal scanning laser cytometry also were used for assessment of mRNA levels and P450-mediated O-deakylation of various resorufin derivatives, respectively. Results with these highly sensitive assays also failed to establish phenobarbital induction capacity in the hepatoma cell lines. Thus, although transformed hepatocyte lines offer the investigator a stable and inexpensive alternative to *in vivo* testing or primary cell cultures, use of these cells needs to be approached conservatively as they may not accurately model biotransformation potential existing *in vivo*. Supported by NIH grants GM32281and ES04696.

T-13 Detection of Human Extrahepatic Cytochrome P450 (CYP) Expression using RT-PCR. F. M. FARIN, M. R. Andersen and C. J. Omiecinski. Department of Environmental Health, University of Washington, Seattle, WA 98195.

The CYP enzyme systems contribute to the bioactivation of various environmental pollutants to potential carcinogenic and cytotoxic intermediates. These enzymes are generally abundant in the liver but also are present in extrahepatic tissues. Thus, local cellular metabolism may well dictate the toxic effects of substances occurring in extrahepatic target organs. Since relatively little is known regarding expression of biotransformation enzyme systems in human extrahepatic organs, studies were conducted to identify mRNA expression patterns of specific CYP in a variety of human tissues including brain, vascular endothelium, foreskin epithelium, oral epithelium and cervical epithelium. Semiquantitative measurements of CYP mRNA expression were determined using specific oligomer probes and RT-PCR. Regiospecific expression patterns of CYP1A1, CYP1A2, CYP3A, and CYP2E1 mRNAs were detected in human brain. Cultures of human umbilical vein endothelial cells (HUVEC) showed constitutive levels of CYP1A1, CYP1A2, CYP2E1, and CYP3A gene expression however, CYP2B6 mRNA was not detected. Interindividual variations of constitutive CYP1A2 and CYP2E1 transcript levels were observed in HUVEC cultures. RT-PCR also demonstrated constitutive levels of CYP1A1, CYP1A2, CYP2E1, and CYP3A in cultures of foreskin, oral and cervical epithelium however, CYP2A6 gene expression was not detection with these assays. Subsequent immunocytochemical and Western blot studies have generally confirmed the presence of certain CYPs in the same tissues. These results demonstrate that human brain tissue, endothelial cells, and squamous epithelium express CYP biotransformation enzymes, and suggest that *in situ* xenobiotic metabolism may be etiologic in various chemically-linked toxicities, including neurotoxicities, vascular abnormalities, and the development of squamous cell carcinomas. Quantitative measurements of CYP mRNAs using RT-PCR are currently being ascertained using synthetic RNA transcripts (rcRNA) for internal RT-PCR standards. rcRNAs are produced from a constructed plasmid containing specific human CYP primer sets and an RNA polymerase promoter. Supported by NIEHS grants ES04696 and ES04978.

T-14 Human Genetic Diversity in Carcinogen Metabolism: Probing Molecular Variation by Polymerase Chain Reaction DOUGLAS A. BELL, Ari Hirvonen, Mary Watson, National Institute of Environmental Health Sciences-NIH, RTP, NC 27709.

Variability or polymorphism in genes that modulate exposure pathways prior to DNA damage appear to be very common and may predispose individuals to environmentally-caused cancer. Functional polymorphisms in carcinogen/drug metabolism genes such as the cytochrome P-450 genes (*CYP*), glutathione transferases (*GST*) and N-acetyltransferases (*NAT1* and *NAT2*) are found at frequencies as high as 60% in some human populations. We have developed a variety of polymerase chain reaction-based (PCR) techniques (including differential PCR, PCR-RFLP, and allele specific PCR) to detect these polymorphisms and the technical aspects of designing PCR methods for genotyping will be presented. We recently reported a PCR-based method for detection of the 5 most common *NAT2* alleles, including a slow-acetylator allele which is found only in individuals with African ancestry and also several new alleles in the *NAT1* gene. We have subsequently determined allele frequencies for *NAT* and other genes in greater than 1500 individuals of European, African, and Asian descent and find significant differences among these populations. *NAT1* genotypes have never been tested in relation to cancer risk. In studies of smoking-induced bladder cancer we find significant risk associated with *GSTM1* and *NAT1* genotypes but not *NAT2*. We observed a significant interaction between a *NAT1* allele and smoking exposure. The combined smoking/*NAT1* genetic risk was greater than 20-fold.

T-15 The Role of Cytokines in Chemical Toxicity

(M. Luster) No Abstract Submitted

T-16 Air Pollutants and Alveolar Macrophage (AM) Function: Relevance of In Vitro Exposures to In Vivo Effects. M.J.K. SELGRADE, Health Effects Research Lab., U.S. EPA, Research Triangle Park., NC 27711

A number of compounds including oxidant air pollutants such as O_3 and NO_2, metals, and volatile organics suppress AM phagocytosis in laboratory rodents following inhalation exposures. In mice, and in some instances rats, extensive work has demonstrated that this suppression of AM phagocytic activity increases susceptibility to challenge with certain gram positive bacteria. Suppresson of AM function has also been demonstrated in human clinical studies following in vivo exposure to O_3. However, for the vast majority of compounds human in vivo exposures are not possible. This study was undertaken to determine whether human and rodent macrophages are similarly affected following in vitro exposure to O_3, and to determine the qualitative and quantitative relationship between effects observed in vitro and in vivo in rodents and humans. In studies to date the levels of suppression of phagocytosis in human and mouse AM following in vitro exposure to the same concentration of O_3 were not significantly different, suggesting that sensitivity of the two species to O_3 is similar. Also, there is at least a qualitative similarity between effects observed in vitro and those observed in vivo. Research is underway to establish more quantitative relationships. It is hoped that this information may then be applied to the interpretation of in vitro human and in vivo animal studies of other toxic air pollutants for which human in vivo exposures are not possible. This abstract does not reflect EPA policy.

T-17 Direct Effects of 2,3,7,8-Tetrachloro-dibenzo-*p* -dioxin (TCDD) on B-lymphocyte Function: Mechanistic Studies and Comparative Studies Between Mouse and Man. M.P. HOLSAPPLE, Toxicology Research Laboratory, Dow Chemical Company, Midland, MI 48674.

The goals of these studies were to further define the mechanism responsible for TCDD-induced suppression of the antibody response and to compare the sensitivity of murine and human lymphocytes to this effect. Because the B-lymphocyte is a primary cell target, we characterized possible effects of TCDD on the B-cell antigen receptor, surface IgM. TCDD suppressed proliferation in response to anti-IgM, and IgM secretion induced by anti-IgM plus lymphokines. A mechanism of action downstream of the receptor was suggested by two observations. First, TCDD had no effect on either the increased expression of surface Ia or the increase in intracellular Ca++ following treatment with anti-IgM. Second, the proliferation of B-cells in response to PMA + ionomycin was suppressed by TCDD. Because the suppression was dependent on the concentration of ionomycin, but not PMA, an important role by Ca++ was suggested. Additional support came from studies which showed that calcium-independent models of B-cell activation (CD40 and PWM) were refractory to TCDD-induced suppression, and from studies with fura-2 which showed that direct exposure to TCDD caused an increase in the levels of basal Ca++ in isolated B-cells. Because we saw no effect of TCDD on calcium influx using radiolabelled ^{45}Ca, the mechanism may involve an effect on an intracellular Ca++ pool. The results suggest that the suppression by TCDD may be mediated in part through an inappropriate elevation of intracellular Ca++. Studies comparing murine splenocyte and human tonsillar lymphocyte cultures indicated that human lymphocytes are sensitive to the direct suppressive effects of TCDD, and that antibody production stimulated by the superantigen, toxic shock syndrome toxin (TSST), may be a useful model to further study the effects of TCDD on human immunocompetence. (Supported by NIH Grant ES 02520)

T-18 The Role of Interleukin-10 in the Induction of Immune Suppression by UV Exposure. S. E. ULLRICH, M. D. Anderson Cancer Center, Houston, Texas 77030.

Exposing mice to ultraviolet (UV) radiation results in a specific, selective suppression of the immune response. The induction of immune suppression by UV has been shown to be associated with skin cancer induction in both experimental animals and humans. How UV induces systemic immune suppression is an intriguing question. The inability of UV to penetrate much past the dermal-epidermal junction of the skin suggests that indirect mechanisms are involved. We have examined the role of epidermal cytokines in the activation of systemic immune suppression following UV exposure. UV exposure induces cultured keratinocytes to secrete biologically active interleukin (IL)-10. Injecting supernatants from these cultures into mice mimics the effects of whole-body UV exposure and suppresses the ability of spleen cells to present antigen to T helper 1 type (Th1) cells. Treating the keratinocyte supernatants with anti-IL-10 reverses the suppressive effect. Furthermore, IL-10 is found in the serum of UV-irradiated mice and spleen cells from mice exposed to UV do not present antigen to Th1 cells. On the other hand, spleen cells from UV-irradiated mice are more efficient at presenting antigen to T helper 2 type (Th2) clones, and injecting anti-IL-10 reverses these effects. In addition, the induction of delayed-type hypersensitivity (DTH) is suppressed in UV-irradiated mice, Injecting antibodies to IL-10 reverses the suppression of DTH.. We suggest that a consequence of IL-10 production by UV-irradiated keratinocytes is to modulate antigen-presenting cell function to cause a shift to a Th2-like immune response. Th2 cells, by secreting cytokines such as IL-4 and IL-10, prevent the further activation of Th1 cells, which suppress cell mediated immune reactions like DTH and tumor rejection.

P-1 Osmotically-induced Plant Defense Genes: Structure and Function. RAY A. BRESSAN. Purdue University, 1165 Horticulture Building, West Lafayette, IN 47907-1165

Both abiotic and biotic stresses cause severe limitations in agriculture. They not only impact yield and quality of crops but are the major reasons for using toxic and carcinogenic chemicals in agriculture, eventually contaminating the food chain and environment. Controlling these stresses by genetic means represents a major step toward alleviating these problems. Much research during the past several years has concluded that genetic control of stress tolerance in plants is complex. In particular it has become clear that the response to osmotic stress and to pathogen attack in plants involves the regulated expression of a number of genes. One of the proteins that has been shown to accumulate in plants in response to osmotic stress is a basic protein termed osmotin. Osmotin is a member of the pathogenesis related gene family 5 and has been shown to have antifungal activity *in vitro* and *in vivo*. The osmotin gene is induced by signals associated with both osmotic stress and pathogens. Therefore, it is clear that the signal transduction pathways that control the expression of genes involved in both abiotic and biotic stress resistance must overlap. We have found that a sequence-specific region (-108/-248) on the osmotin promoter, designated fragment A, is required for a minimal level of gene expression and responsiveness to various external signals. We have demonstrated that fragment A is also sufficient to direct gene expression when fused to a minimal CaMV 35S promoter. Protein factors associated with fragment A were identified in salt-adapted tobacco cells, ABA treated unadapted cells, and young cultured tobacco leaves by gel mobility-shift assays. DNase I footprinting revealed that three conserved promoter elements are involved in DNA-protein interactions on fragment A. These elements are: 1) a cluster of three overlapped G-box-like sequences (G sequence); 2) an AT-1 box-like sequence, 5'-GTATTTTATTAA-3' (AT sequence); 3) a sequence highly conserved in ethylene-induced PR gene promoters, 5'-TAAGTGCCGCC-3' (PR sequence). Among these, the AT sequence was responsible for the majority of protein binding activity of the whole fragment A, whereas the G and PR sequences had weak protein binding activities. Transient expression assays indicated that osmotin promoter activity of these elements correlated with their protein binding activity. UV cross-linking analysis showed that the protein complex bound to fragment A consisted of at least four individual proteins with approximate molecular weights of 28, 29, 40 and 42 kD. Of these, the 28 and 29 kD proteins were associated with the G sequence, and the 40 and 42 kD proteins were associated with the AT and PR sequences. One component of this protein complex, which is associated with the G sequence, is a 14-3-3-like protein.

P-2 Desiccation-induced Protein Synthesis: A Role in Cellular Repair ? M.J. Oliver. USDA-ARS. Plant Stress Unit, Lubbock. TX. 79401.

Desiccation-tolerance is characterized by the ability of plant tissues to survive the air-dried state. Several plant species, ranging from algae to angiosperms, can survive the desiccation of their vegetative tissues.Drying of vegetative cells can be survived by a combination of cellular protection and repair. Plant propagules and desiccation-tolerant angiosperms rely heavily on cellular protection mechanisms, less complex poikilohydric plants apparently rely more on cellular repair initiated on rehydration.These plants thus offer unique models for the analysis of stress induced damage and repair.The desiccation-tolerant moss *Tortula ruralis* is is the most studied of such systems. Early biochemical studies established that protein synthesis is rapidly inhibited by drying indicating that the moss does not have a drying induced gene expression response (i.e., the induction of novel gene products). Analysis of mRNA populations and polysome run-off experiments confirm this. Analysis of patterns of protein synthesis indicates that a change in gene expression is elicited upon rehydration controlled in the main at the level of translation. Patterns are consistent with a repair function for the alteration in gene expression. Analysis of cDNAs representing transcripts preferentially translated during the first 2h after rehydration confirm translational control but also reveal a preferential transcriptional response. But again there are no novel transcripts synthesized during drying. Expression analysis revealed the formation of mRNP particles if drying rates are slow, indicating that the moss stores mRNAs for use when water returns to the system. The formation and nature of these particles is under investigation. Sequence analysis indicates possible repair and protection functions of the rehydration clones.These data will be discussed in relation to the biology of this system and its relevance to higher plant stress research.

P-3 Regulation of environmental stress- and abscisic acid-induced genes

TUAN-HUA DAVID HO, Dept of Biology, Washington Univ, St. Louis, MO 63130

The growth and development of plants are constantly under the influence of environmental stress conditions. Abscisic acid (ABA), whose level is elevated when plants are under stress, plays a pivotal role in stress response. To understand the molecular action of ABA, we have analyzed the promoter of a barley gene, *HVA22*, whose expression is induced by ABA and various stress conditions such as drought, cold and salinity. Using the biolistic transformation/ transient expression approach, we have observed that an ABA response complex consisting of a G-box (GCCACGTACA) and a novel coupling element, CE1 (TGCCACCGG), is sufficient for high-level ABA induction, and replacement of either of these sequences abolishes the ABA responsiveness. We suggest that the interaction between G-box sequences and CE-type sequences determines the specificity in ABA-regulated gene expression. Our results also demonstrate that the ABA response complex is the minimal promoter unit governing high-level ABA induction; four copies of this 49-bp-long complex linked to a minimal promoter can confer more than 100-fold ABA induced gene expression. Besides basic information concerning the action of ABA, this synthetic promoter could be used to regulate the expression of foreign genes whose products could be beneficial to stressed plants.

P-4 Role of the Tobacco Anionic Peroxidase in Growth and Development. L. Mark Lagrimini, Department of Horticulture and Crop Science, Ohio State University, Columbus, OH 43210-1096

Plant peroxidases have long been implicated in such diverse processes as cell wall biosynthesis, auxin metabolism, and host defenses. The tobacco anionic peroxidase promoter was fused to GUS, and its expression localized in transformed plants. The gene shows a specific developmental pattern of expression starting in the youngest leaves in the trichomes, progressing to the epidermis, then the primary xylem parenchyma and the exterior phloem fibers, and in the oldest tissues progressing to the starch sheath and pith. Expression is also seen in floral tissues after fertilization. In the roots expression is limited to the cortical cells at lateral root branch points and the oldest root tissue near the base of the plant. These data support a role for this enzyme in host defenses and lignification in the primary (but not secondary) xylem and the phloem fibers. We have also used the peroxidase/GUS chimeric gene to examine possible regulators of peroxidase gene expression. Of more than 15 treatments, only auxin had an effect on peroxidase gene expression - which was to suppress its synthesis. Transgenic tobacco plants which overproduce or underproduce the anionic peroxidase also support the role for this enzyme in lignification of primary xylem, protection from insect damage, and the metabolism of indole-3-acetic acid. Plants overexpressing this enzyme show many phenotypes typical of low auxin/cytokinin ratio, and plants underexpressing this enzyme show characteristics typical of plants with abnormally high auxin/cytokinin ratios.

P-5 Molecular Aspects of Crassulacean Acid Metabolism: an Adaptation to Environmental Stress.
JOHN C. CUSHMAN, Holly J. Schaeffer and Nancy R. Forsthoefel. Department of Biochemistry and Molecular Biology, 350 Noble Research Center, Oklahoma State University, Stillwater, OK 74078-0454, USA

Crassulacean Acid Metabolism (CAM) is a widely recognized photosynthetic pathway that allows plants to survive conditions of limited water availability. CAM plants conduct the majority of CO_2 uptake and fixation into C4 acids nocturnally when water loss due to transpiration is minimal. Subsequent daytime decarboxylation of the stored C4 acids serves to concentrate CO_2 and limits photorespiration during refixation by Rubisco. Thus, CAM plants exhibit water use efficiencies that are superior to both C3 and C4 plants, a distinct advantage for long term survival under environmental stress conditions, and display energetically favorable rates of CO_2 fixation. The common ice plant (*Mesembryanthemum crystallinum* L.), a facultative CAM plant that switches from C3 photosynthesis to CAM under high salinity or drought stress, is being used as a model to understand the molecular and physiological requirements for CAM. This transition to CAM is accompanied by dramatic increases in the expression of genes encoding enzymes that participate in glycolysis/gluconeogenesis, malate metabolism, and protein turnover. Most of these genes have been cloned and emphasis has shifted towards understanding the coordinate regulation of CAM gene expression by transcriptional activation and plant growth regulators. Using a microprojectile-based transient transformation assay, we have identified specific regions that control salt-inducible gene expression within the promoters of the CAM genes, phospho*enol*pyruvate carboxylase (*Ppc1*) and glyceraldehyde 3-phosphate dehydrogenase (*Gap1*). Current work on the isolation and characterization of trans-acting factors that participate in salt-inducible transcriptional activation, as well as the role of post-transcriptional and post-translational regulatory mechanisms will be discussed.

P-6 Strategies for Dealing with Limitations of Somatic Embryogenesis in Hardwood Trees.
S.A. MERKLE. D.B. Warnell School of Forest Resources, University of Georgia, Athens, GA 30602.

Despite progress over the past decade in initiating embryogenic cultures of diverse hardwood species, application of these cultures for commercial production of superior and engineered genotypes has lagged. While each species presents its own difficulties with regard to application of somatic embryogenesis, some limitations common to many embryogenic hardwood systems are: (1) Low multiplication rates due to low frequency embryo production and/or poor conversion, and (2) The inability to initiate embryogenic cultures from mature trees of known genetic value. With regard to multiplication rates, induction and manipulation of proembryogenic masses (PEMs) has several advantages. Black locust (*Robinia pseudoacacia*) and yellow-poplar (*Liriodendron tulipifera*) PEMs can be manipulated to produce thousands of single, mature somatic embryos with high conversion rates. PEMs are also excellent target tissue for biolistic gene transfer. Treatments known to promote seed germination can also raise conversion frequencies. American chestnut (*Castanea dentata*) somatic embryos require a cold treatment to promote germination and a desiccation treatment to promote apical growth. Accelerated removal of germinated embryos from in vitro conditions can promote plantlet establishment. Ex vitro conversion of *Magnolia pyramidata* somatic embryos produced plantlets with superior vigor to those converted in vitro. With regard to inducing embryogenesis from mature trees, staminate flower parts provide a source of cells with the flexibility to undergo indirect embryogenesis. Sweetgum (*Liquidambar styraciflua*) staminate inflorescence axes generated embryogenic cultures when cultured on a medium containing thidiazuron. Primary somatic embryos produced repetitively embryogenic cultures, providing a route for clonal propagation of the source tree.

P-7 Somatic embryogenesis in *Picea abies*: Morphological and biochemical characterization of various developmental stages.
S. VON ARNOLD, H. Mo and U. Egertsdotter. Uppsala Genetic Centre, Department of Foerst Genetics, The Swedish University of Agricultural Sciences, P.O. Box 7027, S-750 07 Uppsala, Sweden.

Plant regeneration via somatic embryogenesis has practical application in forestry. In addition the process offers unique opportunities to study embryology.

Cortical cells in the primary explant are stimulated to go through repeated divisions so that dense nodules are formed from which somatic embryos differentiate. The first formed somatic embryos continue to proliferate and give rise to embryogenic cell lines which we have categorized into two main groups, A and B. Somatic embryos from group A can be stimulated to go through a maturation process when treated with ABA. Mature somatic embryos can develop into plants.

Group A embryos are characterized by having a large embryonic region, consisting of more than 2000 cells, while group B embryos have less than 300 cells in their embryonic region. In addition to the morphological differences they also differ in growth characteristics such as degree of aggregation of somatic embryos and pattern of proliferation from single cells and protoplasts. The different groups of embryos are also characterized by the amount and pattern of extracellular proteins, such as peroxidases, chitinases, zeamatin-like proteins and AGPs. Our hypothesis is that somatic embryos belonging to group B are less developed than those belonging to group A and that the group B embryos are blocked in their development. Group B embryos can be rescued when treated with seed extract or concentrated extracellular proteins secreted by A embryos. We are now identifying secreted proteins which are developmentally regulated and studying if they also have a regulatory function during embryo development.

P-8 Cellular, Biochemical and Molecular Bases of Stable Maturation-Related Characteristics.
WESLEY P. HACKETT, Depts. of Horticultural Science and Plant Biology, University of Minnesota, St. Paul, MN 55108.

The bases of differential competence for photosynthesis, adventitious root initiation and anthocyanin accumulation in juvenile and mature phase Hedera helix have been investigated. Our results indicate that differential competence for these three characteristics is related to differential gene expression. A chlorophyll a/b binding protein gene is more highly expressed in juvenile than mature leaf lamina exposed to identical environmental conditions. This difference in expression may be related to the relative ability of juvenile and mature leaves to adapt to high irradiance conditions for photosynthesis. A proline-rich protein gene is more highly expressed in mature than juvenile leaf petioles cultured in vitro under conditions that promote rooting in juvenile but not mature petioles. Auxin represses expression in young mature petioles that have some rooting competence but not in old mature petioles that have no competence. In situ hybridization shows that this gene is expressed specifically in cells that are involved in the root initiation process. The lack of competence to synthesize anthocyanin in mature phase leaves and stems is due to the lack of dihydroflavonol reductase (DFR) activity. The lack of DFR activity is due to the lack of transcription of the DFR gene in mature phase tissues.

P-9 Immature Embryo Culture of *Quercus alba*. K. S. GRUMBINE and N. D. Camper. Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377.

Oak species are dominant shade tree species in residential landscape and forested areas. The oak wilt disease is a potentially serious disease in southern oak plantings. In order to understand the nature of the disease and to identify potentially resistant plant material, suitable bioassays are needed. Therefore, studies were initiated to establish optimal conditions for in vitro culture of immature embryos of white oak (*Quercus alba* L.). Acorns were collected, disinfested and embryos removed for culture protocols. Media tested included Murashige and Skoog (MS), Woody Plant Medium (WPM) and Gresshoff and Doy (GD) with different concentrations of napthaleneacetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D) in combination with benzyladenine (BA). After seven weeks, embryos on WPM grew better than embryos on either MS or GD media. Growth and development was better on media with NAA than 2,4-D. NAA stimulated embryo growth whereas 2,4-D stimulated callus development. Concentration effects were minimal with either NAA or 2,4-D. Optimal growth was observed with 1 or 3 mg/L NAA in combination with 1 mg/L BA. A culture protocol for in vitro culture of immature oak embryos has been developed.

P-10 Lower Oxygen and Somatic Embryogenesis of Loblolly Pine (Pinus taeda L). LI, X.Y. and F.H. Huang, 316 Plant Science Building, University of Arkansas, Fayetteville, AR 72701.

Effects of lower oxygen on late pro-embryo development and maturation of somatic embryos of loblolly pine were investigated. The basic culture media and methods followed the US patent No. 5,036,007 (Gupta and Pullman, 1991) except boric acid 31.0 mg/l supplement. Oxygen concentrations at 5% and 10% were used for these examinations. An Oxyreducer (Reming Bioinstruments) connected to a sealed woody chamber was used to control oxygen concentration while the nitrogen used to remove the air oxygen. Compared to the ambient (21%) control, in late proembryo development stage, 10% oxygen promoted the growth of embryogenic tissue, prevented the embryogenic tissue from yellowing within 4-6 weeks and prolonged the subculture period while the 5% oxygen inhibited the growth of embryogenic tissue. The effects of oxygen concentrations 10% and 5% combined with different concentrations of ABA on the maturation of somatic embryos were also investigated.

P-11 Imaging of Fluorescent-stained Somatic and Zygotic Pine Embryos with Laser Scanning Confocal Microscopy. R.NAGMANI, A. Kakani, A.M. Diner*, G. Brown, V. Sapra and G.C. Sharma. Department of Plant & Soil Science, Alabama A & M University, P.O. Box 1208, Normal, Al 35762. * USDA/FS, Southern Forest Experiment Station, Alabama A& M University, Normal, AL 35762.

Embryogenic cultures of longleaf pine *(Pinus palustris* Mill.) were established from zygotic embryos within female gametophytes cultured on MSG and DCR media supplemented with growth regulators. Developing somatic and zygotic embryos were stained with either acridine-orange or ethidium bromide and the whole mount preparations were examined using a Bio-Rad MRC 600 laser scanning confocal fluorescence microscope. A series of thin optical sections (or z series) of fluorescent-stained embryos was obtained, then merged to develop 3-dimensional images. Cells of the embryonal heads of both somatic and zygotic embryos showed greater fluorescence than did those of the suspensor, suggesting greater concentrations of nucleic acids, with concommitantly greater metabolic activity. A group of cells at the embryonal head/suspensor interphase was clearly demarcated by differential staining. A small, central zone of presumptively apical meristem, suggesting initiation of the shoot apex, was oberved in the precotyledonary head. The pixel graph revealed relative rates of tissue fluorescence suggesting specific localized regions of differerent cell/tissue metabolic activity in the embryos.

P-12 Plant Cell Culture: An Alternative for Production of Pharmaceuticals. M.MISAWA, BIO INTERNATIONAL INC.

Decreased plant resources, increases in labor cost and problems associated with obtaining useful substances such as pharmaceuticals from plants have stimulated work in plant cell culture for production of these products. Because plant cell culture is not affected by changes in environmental conditions such as climate or natural depredations, improved production may be available under industrial settings. At present, only two Japanese companies are manufacturing plant products on a commercial scale, but several other products such as taxol seem to be close to commercialization.
In general, however, there are still a few barriers which must be overcome before commercial production of many other products can occur. The production cost is one of the problems because of low productivity of cultured cells. A variety of approaches to improve the productivity of the culture, including optimization of cultured conditions, selection of high-producing cell lines and application of immobilized cells have been tried by many researchers.

Studies on production of several commercially interesting products which are currently being carried out will be reviewed.

P-13 Taxol - The Science and History of an Anti-cancer Compound From US Forests.
DAVE ELLIS, Department of Horticulture, University of Wisconsin, Madison, WI 53760

Taxol, a diterpene produced by *Taxus* spp. has emerged from relative obscurity to an important anticancer compound in only the past two decades. The rapid escalation in the value of taxol is due to its unique mechanism of action as a mitotic block and a persistent campaign by the National Cancer Institute. The only approved source of taxol was the bark of *Taxus brevifolia*, a slow growing sparsely dispersed tree native to the Pacific Northwest of the US. Until recently taxol supply limited both clinical trials and the commercial distribution of the drug. The need for more taxol to both complete and initiate new clinical trials placed the environmentalists and the medical profession at odds over the use vs preservation of the *Taxus brevifolia* trees on national forests. Due to this controversy, as well as the potential impact on ovarian, breast, and other cancers, several major research efforts focusing on identifying alternative sources of taxol were initiated. The semi-synthesis of taxol from precursors, the use of plant parts other than the bark, the utilization of other *Taxus* species, and the production of taxol from suspension and tissue culture are examples of promising alternative sources of the drug. In tissue culture, taxol has been produced from virtually every explant and culture system tested, including suspension, embryogenic, nodule, shoot, embryo, callus and *Agrobacterium* transformed cultures. Although most of these systems produce taxol at levels lower than that found in the plant, the identification of cell lines with a high taxol biosynthetic capabilities and the scale up of these cell lines is ongoing. The elicitation and biotransformation of taxol from several precursors is another area where both increased taxol production and a better understanding of taxol biosynthesis can be obtained. This later point is crucial because as our understanding of processes controlling the production of plant secondary compounds increases, so will our ability to manipulate the production of valuable compounds like taxol.

P-14 Taxol Productivity Of Suspension Cultures Of *Taxus cuspidata* Exposed To Defined Headspace Gas Concentrations. N. MIRJALILI and J.C. Linden Department of Chemical and Bioresources Engineering, Colorado State University, Fort Collins, Colorado 80523

Taxol, an extractive of the *Taxus brevifolia* (Pacific yew), has demonstrated anticancer activity. The goal of our project is to produce taxol in sufficient quantities for clinical use by manipulating cell culture parameters. The parameter of interest for this study is the effect of dissolved gas concentrations on growth and taxol production in suspension cultures of *Taxus cuspidata*. Gas phase concentrations are varied by continuous flow of oxygen, carbon dioxide and ethylene mixtures into shake flasks at 25°C rotating at 125 rpm. The effect of each gas on cell growth and taxol production is investigated using several factorial design experiments. Low head space oxygen concentration (10% v/v) promotes production of taxol prior to day 14. High concentration of carbon dioxide (10% v/v) inhibits taxol production compared to 0.1% (v/v). Taxol concentration is increased as ethylene concentration is increased from 2 to 5 ppm. Maximum taxol concentration in the culture medium correlated with low rates of calcium uptake, high rates of phosphate uptake and adequate fructose for energy production.

P-15 Use of Rotating Wall Vessel (RWV) for Study of Plant Cell Culture. XINSHI SUN and James C. Linden, Department of Chemical and Bioresource Engineering, Colorado State University, Fort Collins, CO 80523.

Plant cells have been difficult to grow in conventional stirred bioreactors. One reason is the hydrodynamic environment. The Rotating Wall Vessel (RWV), which was designed by NASA for mammalian cell culture, shows some prospects for plant cell culture. The system is a horizontally rotated, bubble free culture vessel with membrane diffusion gas exchange. Destructive shear forces are minimized because this system has no impellers, air lift, bubbles or agitators. Taxus cuspidata cells are cultured in a 250 ml working volume RWV for 25 days. Samples are collected every three days. The cell dry weight and concentrations of sugars, calcium and phosphate are determined. Results from growth at different oxygen pressures are also presented.

P-16 In Vitro Culture of Ginkgo. N.D. CAMPER, D.E. Wedge, R.J. Keese and A. Depew. Department ofPlant Pathology and Physiology, Clemson University, Clemson, SC 29634-0377

Ginkgo biloba L. is a native of China and the only living representative of a once large group of plants which flourished for centuries in temple gardens of China and Japan. It is used in the United States and Canada as an ornamental tree. Ginkgo produces a number of chemicals of pharmaceutical importance including the ginkgolides. Mass production of the ginkgo tree and/or providing a source of plant material for harvesting ginkgolides is of commercial interest. The objectives of this study were to optimize the in vitro culture of ginkgo and to evaluate ginklgolide production by cultured plant material. Ginkgo seeds were collected, cleaned and stored at 4 C until used. Cotyledons and embryos were aseptically removed and placed on culture media. Explants were cultured on Murashige and Skoog media with kinetin or benzyladenine and varying concentrations of either 2,4-dichlorophenoxyacetic acid (2,4-D) or naphthaleneacetic acid (NAA). Cultures were incubated at 25 C under a 16-hour photoperiod. Ginkgolide presence in tissue extracts was confirmed by visualization after separation by TLC. NAA was more effective in inducing callus from cotyledonary tissue after 4 weeks than 2,4-D; the optimal level of NAA was 1.0 mg/l. Tissue grown on 2,4-D produced more ginkgolide than tissue grown on NAA. Callus is less organized structurally as compared to cotyledonary tissue and may have contributed to the observed differences in ginkgolide production. Regeneration of plantlets was obtained with embryo culture.

I-1 Factors Influencing Cellular Interactions in the Developing Antennal Lobe of the Moth, *Manduca Sexta*. LYNNE A. OLAND, ARL Division of Neurobiology, Univ. of Arizona, Tucson, AZ 85716.

We have been using primary cultures from the antennal lobes of developing adult moth to explore humoral and direct cellular interactions that may influence the biophysical and morphological differentiation of neurons and glia. Changes in the titer of 20-hydroxyecdysone produced little change in neurite outgrowth while exposure to sensory induced significantly greater branching. Since evidence from previous *in vivo* studies (Oland and Tolbert, 1995) had suggested that interactions between sensory axons and their targets could be mediated by glial cells as well as directly, we have targeted our studies on examining neuron-glial cell interactions. Primary cultures of glia are short-lived, however, so we have developed a slice preparation of antennal lobe that allows us to examine glial cells within the 3-dimensional structure of the lobe. We combine whole-cell patch-clamp recording with dye-labelling to explore morphological development, coupling among glia (and neurons), and responses to neurotransmitters known to be present in the developing lobes. In addition, we are developing a protocol for organotypic cultures. We expect these cultures to give us access to long-term interactions as well as to provide additional clues that may help to define the conditions necessary for long-term survival of glia in primary cultures. [Funded by NIH NS28495.]

I-2 Effect of the CryIA Group of *Bacillus thuringiensis* toxins on midgut epithelial cells and insect cell lines from lepidopteran larvae. D. BAINES[1], J.-L. Schwartz[2], S. MacIntosh[3] and O. Thastrup[4]. [1]Forest Pest Mgmt. Inst., 1219 Queen St. East, Sault Ste. Marie, ONT, Canada. P6A 5M7; [2]Biotechnology Research Inst., 6100 Royalmount Rd., Montreal, Quebec, Canada H4P 2R2; [3]Novo Nordisk-Entotech, 1477 Drew Ave., Davis, CA 95616; [4]Novo Nordisk, Moerkhoej Bygade 28, 2860 Soeborg, Denmark

The susceptibility of many Lepidoptcran larvae to *Bacillus thuringiensis* toxins has been documented. The mode of action of lepidoptcran-specific CryIA toxins is of particular interest as most commercial formulations contain these toxins. We have compared the effects of these toxins on the first target tissue, midgut epithelial cells and insect cell lines (Cf-1, Sf-9, Sec). Midgut epithelial cells were equally susceptible to the CryIA (a,b,c) toxins while the cell lines were susceptible to CryIA (c) using the qualitative Lawn assay. In contrast, midgut epithelial cells were about ten fold more susceptible to CryIA (b,c) toxins than CryIA (a) toxin using the quantitative Live/Dead Assay. The cell lines responded in a similar dose range as midgut epithelial cells for CryIA (c) toxin in both assays, but this was not true for CryIA (a,b) toxins. The cell lines gave no response to these toxins in the lawn assay whereas they did have a differential response to these toxins using the Live/Dead Assay. This suggests that the Live/Dead assay provides a better assessment of the impact of Bt toxins on midgut epithelial cells and insect cell lines. The data show that midgut epithelial cells provide a better model of the *in vivo* response to toxins than the cell lines. We have used these cells to identify physiological mechanisms that could be affected by Bt toxins resulting in insect death. A possible link between Bt toxicity and the disruption of regulatory mechanisms affecting cell volume will be presented.

I-3 Mechanisms of Radiation Resistance in Lepidopteran Insect Cells, T. M. KOVAL, National Council on Radiation Protection and Measurements, 7910 Woodmont Ave., Suite 800, Bethesda, MD 20814.

Cells cultured from insects are considerably less sensitive to ionizing radiation-induced killing than cultured mammalian cells. Furthermore, the degree of radioresistance corresponds to insect order, with lepidoptera being by far the most resistant of the orders examined. The role of DNA repair processes remains somewhat paradoxical in that certain types of DNA repair appear to be associated with radioresistance and other types do not. However, it is increasingly evident that an inducible recovery mechanism is at least partially responsible for radiation resistance in lepidopteran cells. This inducible system is dependent upon transcriptional and translational activities which are not present in unirradiated cells or cells receiving less than some minimal amount of radiation necessary for activation of the process, *i.e.*, the inducible enhancement of survival is abrogated by inhibiting RNA and protein synthesis. Two-dimensional polyacrylamide gel electrophoresis reveals the *de novo* synthesis of several proteins as well as the complete inhibition of others following radiation treatment. Many other proteins are either up- or down-regulated. Transfection studies further indicate that lepidopteran DNA is capable of increasing the radioresistance of radiation-sensitive hamster cell mutants to the level of wild-type hamster cells. These studies suggest a genetic basis for lepidopteran cell radioresistance that involves a carefully choreographed regulation of presently undetermined gene activity in response to radiation exposure.

I-4 A Rotenone-Resistant Cell Line and Its Specific Characteristics. J. MITSUHASHI and Y. Yanagimoto. Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

Rotenone has been known to inhibit in vitro multiplication of cells derived from insects at very low concentrations. Resistance of cells to rotenone was found to be quite variable among cell lines. The degree of resistance differed not only between cell lines derived from different insect species, but also between cell lines derived from different tissues of the same species. In the cell lines derived from the cabbage armyworm, Mamestra brassicae, for example, the cell lines from hemocytes were found to be 1000 times as resistant to the rotenone as the cell lines from ovaries. The IC_{50} of the former cell line was 1.8×10^{-7}M, while that of the latter cell lines was 1.7×10^{-10}M. However, the medium which contained 10^{-9}M rotenone and was used for culture of the hemocyte cell line for 3 days, no longer inhibited the growth of the ovary cell line. The rotenone was also deprived of its cell killing potential by incubating with the supernatant of homogenate of the hemocyte cell line for 3 days suggesting that the hemocyte cell line produced and liberated some rotenone-degradating substance(s) into the medium. The supernatant lost its rotenone inactivating potential when it was heated at 75℃ for 15 minutes, suggesting that some proteinous substance(s) is responsible for the degradation of rotenone. Characterization of this substance is under way.

I-5 Environmental Effects on Oyster Hemocytes in Primary Culture. W.S. FISHER. U.S. Environmental Protection Agency, Environmental Research Laboratory, 1 Sabine Island Dr., Gulf Breeze, FL 32561

Exposure of oyster (*Crassostrea virginica*) hemocytes in primary culture to tributyltin (TBT) demonstrated suppression of specific hemocyte activities related to their immunological functions. Subsequent studies have investigated whether these *in vitro* findings were reliable indicators of decreased hemocyte function *in vivo*. Using continuous-flow exposure systems, oysters were exposed to low doses of TBT for 9 weeks and hemocytes sampled. Significant alterations in their activity were noted. After TBT exposure, oysters were challenged with the protozoan parasite *Perkinsus marinus*. Those oysters previously exposed to TBT suffered higher infection rates and greater mortality. During the challenge period, oyster hemocytes demonstrated further significant alterations in activity. These results are presented in the context of field studies in Tampa Bay FL where *in vitro* assays exhibited activation of hemocyte activities under stress conditions.

I-6 Use of Sponge Cell Cultures as Environmental Indicators of Pollution. SHIRLEY A. POMPONI, Robin Willoughby, M. Edward Kaighn, and He Zhong. US 1 North, Harbor Branch Oceanographic Institution, Inc., 5600 U.S., Fort Pierce, FL 34946

Sponges accumulate chemical pollutants from both seawater and freshwater. Since sponges accumulate heavy metal pollutants in concentrations directly proportional to the degree of pollution, it has been suggested that they may be useful as environmental indicators of water pollution (Richelle-Maurer, Degoudenne, and Van de Vyver, 1994. IN: Sponges in Space & Time, van Soest et al., eds., Balkema, Rotterdam). Our laboratory has developed procedures for cell culture of several species of marine sponges. In addition, we have modified microtiter plate assays to measure esterase activity, protein levels, and DNA concentration to monitor responses of sponge cells to various culture stimuli. Research in progress is correlating physiological responses of sponge cell cultures to various concentrations of pesticide, herbicide, and heavy metal pollutants. This strategy will provide a rapid bioassay procedure for monitoring chemical pollutants in coastal marine environments.

I-7 A SINGLE SECOND MESSENGER MEDIATING OPPOSING GROWTH CONE BEHAVIORS

KATER, S.B., KUHN T.B., SHIBATA A., WRIGHT M.V., WILLIAMS, C.V., Dept. Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523, USA.

Growing neurons encounter both growth promoting and growth inhibitory influences. Some of these influences result in subtle changes in outgrowth (i.e. navigational changes), while others result in dramatic effects (i.e. growth cone collapse). We are dissecting the intracellular mechanisms underlying such differences. Previous work has demonstrated that the growth inhibitory molecule, NI-35, can evoke growth cone collapse via induced rises in intracellular calcium. We have now demonstrated that growth cone contact with oligodendrocytes also results in similar calcium rises. Another set of studies on the growth promoting substance, laminin, also incriminate calcium as a second messenger. How can a single signal, intracellular calcium, direct both permissive guidance responses and growth inhibitory responses in the same neuron? A quantitative comparison of calcium rises in growth cones encountering either oligodendrocytes or NI-35 versus growth cones encountering point sources of laminin sheds light on this issue. Growth cone contact with an oligodendrocyte can produce an order of magnitude rise in intracellular calcium, followed by growth cone collapse. Growth cone contact with laminin coated microscopic beads lead to more subtle changes in calcium, on the order of a 2-fold increase. In addition, NI-35 and oligodendrocyte induced calcium rises occur throughout a growth cone and often spread into adjacent neurites. In contrast, intracellular calcium rises induced by laminin are much more restricted and localized to discrete subregions of the growth cone itself. Taken together, the localization and magnitude of calcium rises provides the major difference by which the same second messenger system can instruct different growth cone behaviors.

I-8 Analysis of Neurogenic Signalling in Cultured Cell Lines from *Drosophila melanogaster*. M.A.T. MUSKAVITCH[1], T.R. Parody[1], S.B. Shepard[2], M. Vaskova [1]. 1, Biology Department, Indiana University, Bloomington, IN 47405; 2, Pathology Department, Faulkner Hospital, Jamaica Plain, MA 02130.

Delta is a cell surface, membrane-spanning signal molecule that interacts with the transmembrane receptor Notch in the fruit fly, *Drosophila melanogaster*. Delta-Notch signalling is required for the correct specification of cell fates in many tissues during embryonic and postembryonic development. We have employed cultured Drosophila cell lines to define properties and activities of the Delta ligand, and to conduct structure-function analyses of the Delta ligand within the context of Delta-Notch signalling. Initial experiments had indicated that Delta interacts heterotypically with Notch on the surfaces of opposing cells, and that Delta can interact homotypically as well. We find that the amino terminus of the Delta extracellular domain includes an EGF-motif binding domain (EBD) that is necessary and sufficient for interaction of Delta with the Notch receptor. Site-directed mutagenesis of the EBD implies that Delta-Notch and Delta-Delta binding activities are structurally separable. A variety of data, including data acquired in cultured cells, imply that activation of Delta signalling requires multimerization, and that the activity of the extracellular Delta EBD is modulated by the intracellular domain of the Delta protein.

I-9 20HE Induced Neuronal Differentiation In Vitro

(Insects) (R. Levine) No Abstract Submitted

I-10 Molecular Analysis of Ecdysone Action in Insect Cells S.R. PALLI, T. Ladd, B. Cook, S.S. Sohi and A. Retnakaran, Forest Pest Management Institute, Sault Ste.Marie, Ontario, Canada, P6A 5M7.

In insects, ecdysone initiates and coordinates molting and metamorphosis through a cascade of gene expression and repression events. Like other steroid hormones, ecdysone acts through intracellular receptors at the transcriptional level. We are using continuous cell lines to understand the ecdysone action at the molecular level. RNA-PCR was used to clone three cDNA fragments, CfEcR (518bp), CHR2 (498bp) and CHR3 (684bp) from spruce budworm, *Choristoneura fumiferana*. These PCR fragments were then used as probes to screen cDNA library for isolating full length cDNAs. These cDNAs were then used as probes on both Northern and dot blots to study the hormonal control of expression of these genes using two continuous cell lines, FPMI-CF-70 (CF-70) and FPMI-CF-203 (CF-203) developed from *Choristoneura fumiferana*. The homologues of CHR2 (MdHR2) and CHR3 (MdHR3) were cloned from IPRI-MD-66 (MD-66) cells using the above described approach and were used to study the gene expression in MD-66 cells. All the three cell lines, CF-70, CF-203 and MD-66 respond to 20-hydroxyecdysone (20E, $4X10^{-6}$ M) in the medium by producing cytoplasmic extensions and clumping of cells within 24 hr. Both clumping and extensions reach a peak between 96-120 hr. At this concentration 20E induces the expression of both CHR2/MdHR2 and CHR3/MdHR3 reaching maximum in 6-12 hr followed by a decline in 24 hr. Cycloheximide does not prevent the induction of CHR2/MdHR2 and CHR3/MdHR3 showing that the induction of these transcription factors is direct. Whereas, repression of CHR2/MdHR2 and CHR3/MdHR3 mRNAs in the continuous presence of ecdysone requires protein synthesis. The overall mechanism of transcriptional regulation by ecdysone will be discussed. Supported by Canadian Forest Service and National Biotechnology Strategy Fund.

W-1 Strategies for Successful Compliance with the Good Laboratory Practice (GLP) Regulations.
D.S. Goldman, Goldman Associates International, Inc., P.O. Box 1853, Rockville, MD. 20849.

The GLP regulations are internationally issued and internationally recognized standards for assuring the quality and integrity of data from nonclinical laboratory safety studies submitted to regulatory agencies (e.g., FDA, EPA) in support of a petition for a research or marketing permit. These regulations deal with the management of laboratory studies and are devoid of impact on or interference with the science of the study. The key issue in the regulations is the insistence on good documentation to show the appropriateness of personnel, facilities and equipment. Full chain of custody of both the test compound and all samples and specimens is required. Full ownership of data, either written or computer captured, is required. All data must be reported and all methodologies must have written acceptance criteria. Accepted standard operating procedures covering all aspects of the study are required. Management, and not the individual scientist, is totally responsible for assuring the quality and integrity of the study data. Management's staff thus includes a quality assurance unit which is independent of the study, monitors the study for compliance, and reports any deviations from compliance to management and the study director who are required to correct any deficiencies noted. Significant penalties are levied against sponsors and testing laboratories in the event of noncompliance with these regulations.

W-2 Application of cGMP Disciplines in Non-regulated Biotech Environments. JOHN L. WEST. Clonetics Corporation, San Diego, CA 92123.

A prerequisite of providing products or services in regulated pharmaceutical and medical device environments is compliance to Good Manufacturing Practices; Minimum requirements for cGMP are provided in 21 CFR 210/211 (pharmaceutical) and 21 CFR 820 (medical devices). Equally, a prerequisite of providing the highest quality goods and services in non-regulated industry is voluntary compliance to cGMP disciplines as an element of the total quality management system. General cGMP disciplines include provisions to define the quality organization structure, facility design, equipment validation and process control, personnel training and documentation requirements, label and batch records control, and handling, storage, control and disbursement of goods. In addition to the obvious economic and quality benefits of compliance to Good Manufacturing Practices for existing markets, the Biotech community, by the nature of its products, is a likely candidate to join regulated industry either as a manufacturer of therapeutic products or as a supplier of goods or services to other regulated manufacturing systems. Ultimately, candidate Biotech companies must install appropriate systems into their operations and pass strict certification audits or lose access to potential markets.

W-3 ISO 9001: The International Challenge. G. KNEBEL. Greiner GmbH, Maybachstrass 2 D-72636 Frickenhausen (Germany).

In the early 1970's the regulatory requirements in Western Europe were covered by a diversity of regulations. Therefore, it was a challenge to establish a general international guideline for quality systems. The ISO 9001,although not specific for any industry, was selected as the international standard for all manufactured devices. To comply with all aspects of an ISO 9001 quality system, it is not sufficient to operate under a sophisticated manufacturing system. The system must adequately document the management policy, and the lines of organization, responsibility, and authority. The main focus of the ISO 9001 is to establish written procedures for design requirements, verification and validation, as well as process procedures, inward control, batch identification, and back traceability of non-conforming products. Operating in a state of quality control and compliance will be a major factor when a company is inspected by international "Notified Bodies" for the purpose of quality system certification. The adoption of a quality assurance system by manufacturers based on the ISO 9001 has improved design and manufacturing procedures, and has benefited the ultimate customer. U.S. pharmaceutical and biotech companies are in the forefront to select ISO certified firms as device suppliers, contractors, and sub-contractors. ISO could be a trade for future domestic sales, as well as a barrier to penetrate the European Market. Most U.S. companies are now turning to the ISO 9001 standard for reliable quality.

W-4 Techniques for Preliminary Bacterial Identification and Determination of Suitable Treatment for Infected In Vitro Cultured Plants. PATRICIA M. BUCKLEY. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

Standard bacteriological techniques are useful for the isolation and preliminary characterization of plant-associated bacteria and for testing their responses to an array of antimicrobial substances. The Gram stain, colony morphology, oxidase test, motility, starch hydrolysis, O/F test, special selective media, temperature, aerobiosis and pH relationships aid significantly in assigning organisms to taxonomic groups. More precise identification may be obtained from carbon source utilization, fatty acid profiling, and cell wall and nucleic acid analyses. Susceptibility of bacteria to antibiotics can be predicted by in vitro disc sensitivity tests and determination of minimal inhibitory and minimal bactericidal levels. Expectation of successful treatment must also include consideration of phytotoxic and environmental effects which can influence markedly the activities of potentially therapeutic substances.

W-5 Indexing Explants and Cultures to Maintain Clean Stock. MICHAEL E. KANE, Environmental Horticulture Department, University of Florida, Gainesville, FL 32611

Combinations of donor plant pretreatment, meristem tip isolation and surface sterilization procedures are routinely used to establish explants *in vitro* which are largely free of surface and endogenous micro-organisms. However, these procedures are not always effective in eliminating surface contamination or infection. Poor aseptic handling can also result in the introduction of micro-organisms into previously uncontaminated cultures. Failure to recognize and eliminate microbial infections can lead to significant losses in commercial micropropagation laboratories. A culture indexing program has been implemented in our research laboratory to routinely screen for cultivable bacterial and fungal contaminants in newly initiated and established stock cultures of more than 60 aquatic, marsh, and ornamental woody plant species. Serial stem slices are inoculated into liquid and agar-solidified Yeast Extract-Glucose, Sabouraud-Glucose, and AC media. Cultures are screened for visible microbial growth following three weeks incubation at 30°C. The reliability and limitations of this procedure will be discussed.

W-6 The Use of Antibiotics in Plant Tissue Culture. GARY SECKINGER, Sigma BioSciences, P.O. Box 14508, St. Louis, MO 63178

Contamination in *in vitro* plant cultures can be a significant problem. Traditionally, tissue culturists have primarily relied on sterile technique to control contamination. The use of antibiotics (and antimycotics) in mammalian cell cultures to control microbial contamination is a common practice. However, their use in plant cell and tissue cultures is considerably less common. Reports on the use of antibiotics for *in vitro* plant applications will be reviewed. The modes of action of various antibiotics and their effectiveness in controlling specific types of contaminants versus their affects on plant growth will be discussed. Technical considerations including storage, handling and use of these compounds for *in vitro* plant research will also be presented.

W-7 Commercialization of Ethylene Regulated Fresh Market Tomato. JOHN BEDBROOK, William Howie, Kathy Lee, Allison Morgan, and Pamela Dunsmuir. DNA Plant Technology Corp., 6701 San Pablo Avenue, Oakland CA 94608

Transwitch™ (sense) constructs of a portion of the ACC 2 gene from tomato under the control of the CaMV 35S promoter in the vector pWTT2144 were introduced into the DNAP proprietary breeding line RG103-114 (a parental large fruited tomato line carrying the og^c mutation). One specific clone, 1345-4 was characterized physically, genetically, physiologically and as a parent in the construction of new hybrid production lines. 1345-4 contained 3 linked copies of the introduced "T" DNA, was completely inhibited for the production of ethylene, and its development was arrested at the "mature green" stage of fruit development. Exogenously supplied ethylene enables 1345-4 to develop to full maturity and the sensory properties of the ripened fruit are indistinguishable from the parental line RG103-114. The agronomic and horticultural properties of 1345-4 are also indistinguishable from the parental line. 1345-4 and hybrids derived from it have been delisted by APHIS and deemed equivalent to the parental lines by FDA. We have used 1345-4 as a parent in the production of hybrid lines which we have commercialized under the trade name of "Endless Summer Tomato". The development of these lines, their approvals by the regulatory agencies, the commercial value and the issues presented by these lines in commercial distribution will be discussed.

W-8

Development of Virus Resistant Cucurbits Through Coat Protein Gene Expression

H.D. Quemada, D.M. Tricoli, K.J. Carney, J.F. REYNOLDS, R.Z. Deng, P.F. Russell, J.R. McMaster, M.L. Boeshore, D.W. Groff, K. Hadden, B. Moraghan, Experimental Plant Genetics, Asgrow Seed Company

Production of squash (*Cucurbita pepo*) and melon (*Cucumis melo*) is compromised by a number of viral diseases. We have employed the strategy of coat protein based protection to produce virus resistant squash and melon hybrids. The virus diseases we have targeted are cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus II (WMVII). We have utilized *A. tumefaciens* and a binary based vector system to move single or multiple virus coat protein genes into a number of cucurbit species. Seed was produced in these species and evaluated in multiple greenhouse and field trials. We will describe the results of these trials and the progress we have made in moving these virus resistant vegetables towards production of commercial hybrids.

W-9 Benefits of Transgenes on the Processing Quality of Tomato (E. Green) No Abstract Submitted

W-10 The Next Generation of High Quality, Genetically Improved Tomatoes. OAKES, J.V., McGuire, C.M., Shewmaker, C.K., Sheehy, J.A., Sanders, R.A., Hiatt, W.R. and Sheehy, R.E. Calgene Fresh, Inc. 1920 Fifth St. Davis, CA 95616

Flavr Savr™ tomatoes are currently being marketed in the United States. These tomatoes have been transformed with an antisense polygalacturonase (PG) gene for delayed softening and increased durability. The next generation of genetically improved tomatoes will contain the antisense PG gene and an antisense 1-aminocyclopropane -1-carboxylic acid synthase (ACCS) gene for delayed ripening and decreased perishability. Results of field trials of these tomatoes will be discussed.

W-11 Commercialization of Beetle Resistant Potatoes. M.A. HINCHEE, New Agricultural Products/Monsanto, 700 Chesterfield Parkway N., GG4I, St. Louis, MO 63198.

New Leaf™ potatoes are nearly ready for commercial sale. These plants contain the Btt gene from *Bacillus thuringiensis* which gives excellent control of Colorado Potato Beetle. New Leaf™ potatoes have undergone several years of extensive field testing across the potato growing regions of the United States and Canada, and are now being evaluated by U.S. regulatory agencies prior to their commercial launch. The consultation with the U.S. Food and Drug Administration under their food policy guidelines has been completed. The USDA has given non-regulated status to these potatoes. The Environmental Protection Agency has concluded a special scientific advisory panel to discuss the issue of insect resistance development. The EPA has granted a limited registration for plant propagation and is in final consideration of a full registration and exemption from the requirement of a tolerance for the Btt protein. The selectable marker NPTU has already received exemption from the requirement of a tolerance. The development, testing, and regulatory approval processes for this important new product will be discussed.

W-12 Enhancing Disease Resistance in Vegetable Crops. J. M. JAYNES, Demeter Biotechnologies, Ltd., Brightleaf Square, Suite 19-D, 905 West Main Street, Durham, NC 27701.

A number of novel molecules (Peptidyl MIMs, short for peptide-based Membrane Interactive Molecules) have been developed which possess the ability to directly destroy many different types of bacterial and fungal plant pathogens. Six to ten log killing of the pathogens can occur *in vitro* at levels found not to be injurious to plant tissues (in many cases at sub-micromolar concentrations). Synergistic killing is also observed when the MIMs are combined in treatments containing lysozyme, chitinase, or other hydrolytic enzymes. Genes encoding several of the more potent MIMs, under the control of a wide variety of both constitutive and non-constitutive promoters, have been introduced into a number of plant species, including several vegetable crops. In further experimentation, some of these transgenic lines have been shown to produce levels of peptide sufficient for them to exhibit enhanced disease resistance properties when challenged with pathogenic micro-organisms. Little oral toxicity to animals is anticipated since high amounts of purified MIMs, when ingested, are very quickly broken down to non-active fragments.

W-13 Apoptosis in an Insect Cell Line: Analogies and Contrasts with Vertebrate Apoptosis.

R.J. CLEM, J.M. Hardwick, and L.K. Miller*. Neurovirology Laboratories, Johns Hopkins School of Medicine, Baltimore, MD 21287-7681 and Departments of Genetics and Entomology, University of Georgia, Athens, GA 30602*.

If given the proper stimulus, cells of the lepidopteran cell line SF-21 (or its derivative SF-9) will die by apoptosis, a type of cell death commonly seen in vertebrates. Apoptosis of SF-21 cells is morphologically similar to vertebrate apoptosis, with features including plasma and nuclear membrane blebbing, chromatin condensation, and preservation of cytoplasmic organelles. In addition, SF-21 cells undergo the classic oligonucleosomal chromatin cleavage which is often associated with vertebrate apoptosis. While apoptosis was morphologically described over 20 years ago, the genetic pathways leading to the induction or inhibiton of apoptosis are only beginning to be defined. Two unrelated families of baculovirus genes are known to block apoptosis in SF-21 cells, *iap* and *p35*. The *iap* genes encode motifs which suggest they may function as transactivators, including a ring (zinc) finger. Although *p35* can prevent the death of mammalian neuronal cells, genes which can in many situations block apoptosis in mammalian cells (Bcl-2 and Adenovirus E1B) do not function in SF-21 cells. Nonetheless, the human ICE protease can induce apoptosis in either mammalian or SF-21 cells. Thus, it appears that the pathways governing apoptosis are for the most part conserved between these evolutionarily divergent groups of animals, although additional interesting differences may emerge upon further study.

W-14 Nonradioactive Methods for Measuring Cell Death in Cell Populations and Individual Cells.

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Traditional methods for studying apoptosis and/or cell-mediated cytotoxicity involve the use of radioisotopes such as ^{3}H, $^{51}Chromium$ or ^{75}Se or dyes such as ethidium bromide. It is now possible to obtain quantitative data without radioactivity by using one of three kits from Boehringer Mannheim: 1) the Cell Death Detection ELISA is based on the isolation and measurement of nucleosomal DNA fragments from cell lysates, 2) the Cellular DNA Fragmentation ELISA is used with proliferating cells to measure BrdU-labeled DNA fragments from either cell lysates or cell culture supernatants, 3) the Cytotoxicity Detection Kit (LDH) is a familiar assay now available in a convenient and easy-to-use format that measures lactate dehydrogenase activity released into cell culture supernatant. In addition, three new kits for use in flow cytometry, fluorescent microscopy or light microscopy make doing the TUNEL assay (3' OH end labeling of DNA strand breaks using terminal transferase and fluorescein dUTP +/-anti-fluorescein antibodies conjugated to AP or POD) allow the distinction between apoptosis and necrosis in individual cells.

W-15 Transformation of Grape (*Vitis vinifera* L.)

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Transgenic grape vines were regenerated from somatic embryos produced both from immature zygotic embryos of two seedless grape selections and from leaves of in vitro grown plants of the cultivar Thompson Seedless. Somatic embryos were bombarded with gold microparticles using the Biolistic PDS-1000/He device (Bio-Rad Laboratories) and then exposed to engineered *A. tumefaciens* EHA101 (E. Hood, WSU). Alternately, somatic embryos were exposed to *A. tumefaciens* without prior bombardment. Following cocultivation, secondary embryos were allowed to proliferate on Emershad/Ramming proliferation medium (Plant Cell Repts. 14:6-12, 1994) under kan selection. Transgenic embryos were identified after 3-5 months and developed into rooted plants on Woody Plant Medium containing 1 µM 6-benzylaminopurine, 1.5% sucrose, and 0.3% activated charcoal. Seedless selections were transformed with plasmids pGA482GG (J. Slightom, Upjohn) and pCGN7314 (Calgene) which carry GUS and NPTII genes. 'Thompson Seedless' was transformed with pGA482GG and pGA482GG/TomRSVcp-15 (D. Gonsalves, Cornell U) containing the tomato ringspot virus coat protein gene. Integration of foreign genes into grapevines was verified by growth on kan, GUS and PCR assays, and Southern analyses.

W-16 Pea Transformation

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As the production of transgenic plants moves fro the experimental to production mode, a high efficient transformation system becomes even mo necessary. The use of gene suppression technologi with their 2-50% efficiency combined with the ne to select transformants with the correct combinati of expression level, T-DNA copy number a complexity also calls for high efficiency. A typic gene introduction involving sense suppression wou require a minimum of 100 transformants, preferab 500. While these numbers are obtainable from a fe experiments in the model tobacco, tomat *Arabidopsis* and *Petunia* systems, recalcitrant cro such as the legumes, the monocots and many woo species, either are not yet transformable or do n have the necessary efficiencies.

Using pea as an example, this talk will illustrate t steps involved in developing a transformation syste for a recalcitrant species. Areas covered will explant selection; development of a regenerati system; choosing a gene, selectable marker and ge delivery system, and will briefly discuss t economics and logistics of transformant producti on a large scale.

W-17 High Efficiency Transformation and Regeneration of Transgenic Sweetpotato Plants.

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Transgenic sweetpotato (*Ipomoea batatas*) plants with a stable and high expression of introduced genes have been developed. Factors that contributed to this success were: the use of highly regenerative sweetpotato genotype (PI 318846-3), an efficient and rapid somatic embryogenesis protocol, an appropriate *Agrobacterium tumefaciens* strain (EHA 101), an appropriate binary vector (with *gusA:nptII* fusion gene driven by double CaMV35S promoter with AMV translation enhancer), and the optimized cocultivation and selection conditions. Leaf and petiole explants, cocultivated with bacterial culture for 3 days, were incubated for 14 days on MS medium with 2,4-D (2.5 mg/l) and 6-BAP (0.25 mg/l). Transgenic embryos were selected on kanamycin-medium (100 mg/l) with ABA (2.5 mg/l), germinated in a basal medium, and plants subsequently transferred to the greenhouse. All the plants showed a strong ß-glucuronidase activity in the histochemical assay. The GUS expression was high in most organs of the mature plants although there was a degree of differential expression. PCR analysis provided evidence of the introduced genes.

W-18

Plastid transformation: A new tool for basic science and for biotechnological applications.

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The circular, 120- to 180-kb genome of plastids is present in 500 to 10,000 copies per cell. Introduction of foreign genes into the plastid genome is achieved by targeted gene insertion. The gene of interest is cloned next to a selectable marker gene which is flanked by plastid DNA targeting sequences in a plasmid vector. Biolistic transformation is followed by integration of the linked transgenes and elimination of the wild-type plastid genome copies during repeated cell divisions. Efficient transformation protocols rely on selection for a chimeric *aadA* gene encoding spectinomycin resistance (1, for review see ref. 2).

Plastid transformation has been applied to study plastid biology (3), gene regulation (4) and RNA editing (5). The plastid genome is also an attractive target for crop engineering due to the potential for expressing the transgenes at a high level. A good example is accumulation of the *Bacillus thuringiensis* protoxin to 3-5% of the soluble protein in tobacco leaves from a plastid-encoded *crylA(c)* gene (6). Efficient containment of the transgenes is an additional advantage in crops in which plastids are not transmitted by pollen. Plastid transformation so far has been reported only in tobacco. After appropriate adjustment of the protocols, applications to other land plants will undoubtedly follow.

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W-19 *Agrobacterium*-mediated gene transfer to rice (*Oryza sativa* L.)

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An important pre-requisite for the successful production of transgenic rice plants is to transfer T-DNA at high frequency to tissues capable of embryogenic response. A system enabling efficient plant regeneration through somatic embryogenesis from in vitro cultured immature inflorescence of rice has been developed by using MS basal medium coordinated with the proper combination of growth regulators. The embryogenic system has been successfully utilized in *Agrobacterium*-mediated gene transfer. The protocol developed by our laboratory relies on infiltrating *Agrobacterium* into various rice tissues with the help of vacuum (30 mm Hg) prior to the co-cultivation and can result in T-DNA delivery at very high frequencies to different explants of rice. The frequency of T-DNA transfer was analyzed using an intron-gus gene as a reporter system. Our results on the patterns of transient and stable gene expression following *Agrobacterium*-mediated gene delivery to immature inflorescence explants and on the molecular characterization of the putatively transgenic rice plants will be presented.

W-20 The Components of Variation Associated with *Agrobacterium*-Mediated Transformation of Soybean.

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The efficiency of delivering foreign genes into the cultivated soybean is relatively low in comparison to other plant species. We currently employ shoot organogenesis from a cotyledon explant, in conjunction with *Agrobacterium tumefaciens*, for plant gene transfer into soybean. We have observed significant genotype variation in transformation frequencies with this system. Genotype variation can be partitioned into two components, *Agrobacterium* susceptibility and the regeneration potential of the genotype. An important aspect associated with susceptibility of the cotyledon explant to *Agrobacterium* infection is the targeting of cells competent for regeneration. The regeneration potential can be subdivided into two phases, shoot initiation and subsequent elongation. We currently are focusing our efforts on optimization of *Agrobacterium* infection and regeneration components to ultimately enhance transformation frequencies with this system.

W-21 Possible Factors Affecting Fertility of Soybean Plants from Transgenic Embryogenic Cultures. W.A. PARROTT, C.N. Stewart, and M. Anis. Dept. of Crop & Soil Sciences, Univ. of Georgia, Athens, GA 30602

It is possible to generate large numbers of independently transformed embryogenic lines of soybean, but these tend to fail to convert, or produce plants that are partially or completely sterile. These problems are generally avoided with protocols that minimize time in culture and promote the rapid recovery of healthy plants. Factors that facilitate plant recovery from embryogenic cultures include an auxin removal treatment for the developing embryos, and differentiation, maturation, and germination steps without exogenous growth regulators. A desiccation step prior to germination, and a photoperiod long enough to prevent premature induction of flowering, also contribute to the recovery of normal plants from about 40% of our somatic embryos. In our hands, sterility has been obtained only in plants recovered from the transformation process. Our fertile transgenic plants have all come from young cell cultures. This indicates a need for methods that permit the rapid establishment of new cell lines. A cryopreservation system would also assist in the maintenace of young cell lines. We have also associated fertility with cell lines that appear 4-6 weeks after particle bombardment. Cell lines that take longer than this to appear may not convert, or will give rise to sterile plants. Finally, transgenic plants with lowered fertility can have extra chromosomes. Examination of embryogenic cultures reveals that 5-10% of the cells are aneuploid. Hence avoidence of unnecessary stress factors which could increase the incidence of aneuploidy may be helpful. The fertility of normal transgenic plants can be stable, or can decrease over generations, in which case crossing to a non-transgenic plant is necessary.

W-22 Establishment of a Regional Soybean Tissue Culture and Genetic Engineering Center. R.D. DINKINS, R.S. Torisky, R. Di, and G.B. Collins. Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091.

We have established a soybean genetic engineering center with the objective of moving useful foreign genes into soybeans. The involvement of three laboratories in the Center, namely the University of Kentucky and collaborators W.A. Parrott at The University of Georgia and J.A. Finer at The Ohio State University has allowed for fast exchange of information addressing and solving problems that are impediments to regeneration of fertile transgenic plants. Each of the three laboratories will follow common procedures to regenerate transgenic soybean plants via somatic embryogenic suspensions. In addition, each laboratory will also concentrate on optimizing procedures that reflect the strengths of the laboratory. Our laboratory at the University of Kentucky has had success in generating transgenic embryo lines using hypocotyl/radicles of mature somatic embryos as targets for DNA introduction by particle bombardment (DuPont PDS-1000). The advantages of this technique, which we call somatic embryo cycling, is that the somatic embryo hypocotyl/radicles are highly embryogenic, and the embryos are initiated from single epidermal cells. The major problem with this method is that transgenic lines are difficult to convert to fertile plants. Experiments are in progress to determine if this is due to the effect of the selectable marker gene used, the extended length of time that the cell lines are in culture, or that the cycling procedure itself leads to inherent problems that cause conversion failure. We are also using the cotyledonary node system to introduce genes using *Agrobacterium*. Using this method we have successfully produced fertile transgenic plants containing genes encoding viral coat proteins. Fertile R_2 plants have been tested successfully for virus resistance.

W-23 Cotyledonary Node Explants of Northern-Adapted Soybeans as Targets for *Agrobacterium*-Mediated Transformation. P.A. DONALDSON, D.H. Simmonds, and H. Voldeng. Plant Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario Canada K1A OC6

Cotyledonary nodes of short-season, northern-adapted soybeans were used as targets for *Agrobacterium*-mediated transformation. Preliminary screening of Plant Research Centre lines confirmed both the presence of an *in vitro* regeneration response of cultured cotyledonary nodes of young seedlings and a high frequency of susceptibility to infection by w.t. *A. tumefaciens* A281 (>C58>ACH5) in whole plant inoculations. Following co-culture with *A. tumefaciens* EHA105 (disarmed strain based on A281) carrying the binary vector pBI121, kanamycin-resistant sectors were recovered at a rate of 10-15% of cotyledonary explants, but most of these arose from non-regenerable portions of the explant. Transformed organogenic sectors were recovered at a much lower frequency (<1%) but lead to recovery of a fertile transgenic plant in which the presence of both *nptII* and *uidA* were confirmed by Southern analysis of the primary transgenic and the T1 plants. Recent results of destructive histological β-glucuronidase assays of explants co-cultured with EHA105 carrying 35SGUSINT, however, suggest that depending on the cultivar and conditions used, the frequency of transformation in regenerable portions of the explant is actually much higher (10-20%). We are examining factors such as dissection technique, pre-co-culture conditions and a modified screening/selection/subculture approach in attempts to enhance identification and survival and regeneration from these transformed sectors.

W-24 Transformation and Transformation-competence in Embryogenic Tissue of Soybean. JOHN J. FINER, Elaine R. Santarem and Harold N. Trick. Horticulture and Crop Science, The Ohio State University, Wooster, OH 44691.

Somatic embryos and embryogenic tissue of soybean can be obtained following culture of immature cotyledons on a medium containing 40 mg/l of 2,4-D and 6% sucrose. Induced embryogenic tissue can then be used to establish proliferative suspension cultures. As with any proliferative tissue culture system, embryogenic suspension cultures of soybean undergo changes over time. These changes may be mediated by the subculture medium, the mode of subculture and the level of visual selection used by the individual performing subcultures. With soybean, suspension cultures appear to go through phases of maturation or stability. Newly-established suspension cultures are quite heterogeneous and contain both proliferating and developing embryos. As the cultures are selected and subcultured, clumps of tissue become more homogeneous and responsive to particle bombardment-mediated transformation. Unfortunately, sterility problems are prevalent in plants regenerated from older cultures (see Parrott et al.). Histological and morphological analyses of transformation-competent older cultures and non-competent younger cultures revealed differences in clump structure and the surface cells within the clumps. Efforts are underway in the laboratory to characterize transformation-competent cultures in an attempt to obtain this phenotype in younger cultures. Our efforts to develop alternate transformation procedures using both the particle gun and *Agrobacterium* with newly-established or induced embryogenic tissues will also be presented.

W-25

Improvement of Regeneration of Nontransgenic and Transgenic Plant Tissues Using a Concentrate Liquid Medium. J.-J. LIN, R. M. Fike, and N. Assad-Garcia. Molecular Biology R&D, Life Technologies, Inc.(GIBCO BRL) 8717 Grovemont Circle Gaithersburg, MD 20878

A convenient concentrate-prepared MS complete liquid medium has been formulated. Preparation of MS medium is achieved by simply adding three components of concentrate-prepared MS liquid medium and the sterilized water together. The requirements of sterilization by autoclave and the adjustment of pH are eliminated. The fresh weight of tobacco suspension cells and calli obtained from the cells grown in the medium prepared from the concentrate-prepared MS complete liquid medium increases 20-30% over those grown in the medium prepared from the MS complete powder medium. The regeneration of shoots from tobacco leaf discs grown in the membrane based containers with the liquid medium made from MS complete concentrate-prepared liquid medium increases 60-70% in the fresh weight and 50% in total number of shoots over those grown in the petri dish with the solid medium prepared from MS complete powder medium. The transgenic shoots containing *gus* gene were regenerated from *Agrobacterium tumefaciens* infected tobacco leaf discs using the membrane based container with the liquid medium made from MS concentrate-prepared liquid medium. In addition, the increase of regeneration of shoots in both fresh weight and the total number of shoots using MS complete concentrate-prepared medium were also observed. The application of MS complete concentrate-prepared medium in different crops such as tomato, *Arabipdosis* and potato will be discussed.

W-26 Advantages of Microporous Membranes for Plant Tissue Culture on Liquid Media.
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Microporous membrane structures that support tissue at the interface between liquid and gaseous phases can allow a number of advantages over semi-solid (agar) and liquid shake flask or bioreactor systems. These advantages include: (1) filter sterilization of media without autoclaving, (2) simple monitoring and alteration of media composition during culture, (3) uniform availability of nutrients and diffusion of exudates, (4) growth of shoots in a gaseous atmosphere, (5) simplified shipping of cultures and handling of tissues within the lab. In addition, membranes incorporated into culture vessels create microbial barriers to preserve asepsis in the vessel while allowing gaseous exchange between headspace in the culture vessel and air in the growth room.

We will discuss these advantages and provide examples in the micropropagation of several crops. In addition, we will introduce a novel membrane based microhydroponic system which incorporates the aforementioned features.

W-27 Plant Micropropagation in Bioreactor Cultures.
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Plant regeneration in liquid cultures through organ or embryo development can be exploited for automation and large-scale bioreactor cultures. Automation can provide a solution to some of the limitations imposed by labor intensive, high production costs of existing conventional technologies. Liquid cultures were used for the propagation of ornamental, vegetable, fruit and woody plant species. Leaf hyperhydration and shoot malformation in liquid media was controlled by the levels of growth promoting and growth inhibiting substances which reduced the shoot system to meristematic or bud clusters. Growth retardants, which are inhibitors of gibberellin biosynthesis, induced meristemoid clusters in Brodieae, gladiolus, Nerine, Lilium, Ornithogalum and celery, and condensed bud clusters in potato, banana, poplar and philodendron. Cluster biomass increase in bioreactors was 3-5 times higher than in shake cultures. The rate of aeration and circulation as well as sucrose and growth regulator levels effected cluster growth and proliferation. The proliferating clusters were separated manually or mechanically for singulation, depending on the species. The propagules were either recycled to bioreactor cultures for further biomass growth or to a second stage media for initial bud growth prior to the transfer to agar cultures for plant development. Potato and plants producing bulbs or corms were induced to form storage organs from the developing buds on the clusters in a species specific induction medium. The importance of liquid cultures for controlled plant development and efficient propagule production will be discussed.

W-28

Liquid Culture As A Route For High Efficiency Micropropagation

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The efficiency of micropropagation can be increased by shortening the time that cultures remain in the growth room, by increasing the multiplication rate of the cultures, and by reducing the labor associated with separation of the multiplication cultures.

Firstly, we will discuss a micropropagation system using interfacial membrane rafts floating on liquid medium to obtain increased shoot proliferation. Using this system for micropropagation of *Aconitum napellus*, a proliferation rate 45% higher than the maximum value on semi-solid medium was obtained. Similar results were obtained with *Spathiphyllum* and *Syngonium*.

Secondly, we will discuss a system of multiplication of shoot clusters in bioreactors equipped with a filter device to control contamination in the proliferation medium, and subsequent mechanical separation of these clusters. In this air lift bioreactor system, mechanically separated shoot clusters of *Syngonium* grown in filtered medium produced 19.5 shoot initials per gram fresh weight of inoculum after 30 days of culture, compared to 8.7 shoot initials in unfiltered medium. Methods for developing compact clusters of meristems (meristemoids) in bioreactor will be discussed, as well as the separation of these meristemoids by use of a dicing cutter.

V-1001

Extracellular Matrix Modulation of the Cellular and Molecular Aspects of Myogenesis. G. Bahador, A. Davalos and S. BENSON. Department of Biological Sciences, Calif. State University, Hayward. 94542

The role of the extracellular matrix (ECM) in the cellular and molecular events of myogenesis is an unresolved question. We have used the specific inhibitor of lysyl oxidase, β-aminoproprionitrile (BAPN), to inhibit collagen crosslinking and studied the effect on in vitro myogenesis. Rat L_6 myoblasts were induced to differentiate and cellular and molecular aspects of myogenesis were evaluated. Cell fusion, myotube formation and CPK activity is inhibited by concentrations of BAPN which do ot effect cell viability and proliferation. The inhibitory effect of BAPN is reversible and time dependent. Interestingly the genes for myogenenin α-actin and myosin heavy chain are expressed at equivalent levels in controlfused and BAPN treated cultures. Curiously, while MHC mRNA accumulates in BAPN treated cultures, the accumulation of MHC protein is greatly reduced compared to control cultures. suggesting translational regulation of mRNA by the ECM. We conclude that an intact ECM is necessary for cell fusion and myotube formation but not for expression of muscle specific genes.

V-1002

Expression of Type VI Collagen During Glioblastoma Cell Invasion in Brain Tissue Cultures. J. Han and J.C. DANIEL. College of Dentistry, Oral Biology Department, University of Illinois at Chicago, Chicago, IL 60612.

Glioblastoma features a large expanding mass which infiltrates gray and white matter in vitro. Little is known about interactions between human glioma cells and normal brain tissue. In this study brain tissue cultures have been used to examine this interaction. Our results with human glioblastoma U-87 MG cells and normal brain tissue fragments indicate that the extracellular matrix protein, type VI collagen, is involved in the invasive process. Tumor cells and solid strands of cells penetrate into the brain tissue. Immunofluorescence data reveal that type VI collagen is associated with the invading glioblastoma cells. We have demonstrated that type VI collagen is involved in glioblastoma cell process extension and penetration in collagen gels in vitro. Tumor cell infiltration and invasion enhanced by extracellular matrix proteins may present a mechanism of tumor invasiveness. This brain culture system is promising for the direct study of interactions between glioblastoma cells and normal brain tissues and for understanding the role played by type VI collagen in invasiveness. This brain culture system also provides a rapid in vitro model with which cellular biological determinants of invasiveness may be studied. Once we understand more of the complex process of tumor cell invasion in brain tissue we may be able to find new ways to control spreading of glioblastoma cells within the brain.

V-1003

Application of a Composite Skin Graft to Nude Mice: Dynamic Interaction between Cells of the Epidermis and the Dermal Graft. E.S. GRIFFEY[1] and S.A. Livesey[2]. [1]Baylor College of Medicine, Center for Biotechnology, The Woodlands, TX 77381 and [2]LifeCell Corp., The Woodlands, TX 77381.

Nude mouse studies were performed to examine the feasibility of grafting an acellular dermal matrix (AlloDerm®) in combination with human neonatal foreskin keratinocytes (HFK). The goal of this research is to enable grafting of full thickness wounds while avoiding the morbidity often associated with skin graft donor sites. AlloDerm has been used clinically to allow the use of a thinner STSG resulting in less trauma to the donor site. However, replacing the STSG with an in vitro reconstituted skin would preclude the need for creating a donor site. Several different techniques were applied to deliver HFK to AlloDerm including simultaneous and delayed application of cell slurries and cell sheets or in vitro reconstituted skin (HFK cultured onto AlloDerm prior to grafting). We have been able to show retention of HFK at 21 days post grafting (using antibodies to human MHC class I, and involucrin) in the co-culture technique. Regardless of the techniques applied to deliver HFK, we noted a dynamic interaction between epithelial cells (human or mouse) and the dermal component of the graft. This is evidenced by a fully differentiated epidermis with rete ridge formation in areas above AlloDerm. In contrast, areas of epidermis above granulation tissue in the absence of AlloDerm exhibited a thinner epidermis and a flat dermal/epidermal junction. At present, the reasons for this interaction are not clear, but may involve specific interactions of keratinocyte receptors with components of the BMC or cytokines released by stimulated host fibroblasts infiltrating the dermal matrix. This research was supported by the National Science Foundation under award number III-9361046.

V-1004

A novel three-dimensional liver culture system with applications to transplantation and extracorporeal liver assistance. B. SIBANDA, J. Gee, J. San Román, V. Kamali, and B.A. Naughton. Advanced Tissue Sciences Inc., La Jolla, CA 92037.

Co-culture of hepatic parenchymal cells (PC) with cells of the hepatic microenvironment (Kupffer cells, endothelia, fibroblasts, fat storing cells, *etc.*) on three-dimensional templates has certain advantages over monolayer-based cultures or cultures of PC on or entrapped within biomatrices such as collagen gels. These include long-term expression of liver specific proteins and cytochrome P450 enzyme activity, and proliferation of PC in association with stroma on the three-dimensional templates. This method was modified so that liver cell co-cultures could be grown on bioresorbable, polyglycolic acid (PGA) felt templates and transplanted. In partially hepatectomized (Hx) rats, co-cultures that were transplanted into the omentum or mesentery developed into organized hepatic tissue within 30 days. However, constant hepatotrophic stimulation was required to maintain this effect; atrophy of the grafts occurred after liver regeneration was complete. In order to circumvent this problem, co-cultures were grafted into the livers of Dalmatian dogs at the time of partial hepatectomy in an attempt to ameliorate the congenital hyperuricemia found in this animal. Co-cultures were generated using Beagle PC (exhibiting normal uric acid transport) and Dalmatian stromal cells. Dalmatians were immunosuppressed with cyclosporine. Urine uric acid levels of dogs receiving the co-culture grafts were consistently below those of controls for up to 70 days post transplant indicating that the liver regenerated as a chimera of Dalmatian PC and the Beagle PC which metabolized the uric acid. This method may prove useful in treating hepatic metabolic diseases arising from single gene defects. Another application that is currently being evaluated is the use of liver co-cultures generated by this methodology in an extracorporeal device to provide liver assistance to animals following lethal-Hx or chemically-induced hepatic failure.

V-1005 A Method for the Primary Culture of Epithelial and Stromal Cells from Normal Rat Dorsal Prostate and from Rat Prostate Carcinomas. M.S. CONDON and M.C. Bosland. New York University Medical Center, Nelson Institute of Environmental Medicine, Long Meadow Road, Tuxedo, NY 10987.

The objective of this work was to develop a reliable method for the isolation and culture of epithelial and stromal cells from the normal rat dorsal prostate and from rat prostate carcinomas for coculture studies of cells derived from the *same* tissue. Two distinct cell isolation procedures were developed, both using an explant-outgrowth approach; one encouraged epithelial growth, while the other favored stromal growth. Plating of explants of normal prostate tissue on polystyrene dishes in Ham's F-12K with 5%FBS, 50nM testosterone, penicillin, and streptomycin (YD medium) resulted in virtually pure cultures of fibroblasts. For the selection of normal epithelium, Prostate WOW medium was developed, consisting of Ham's F-12K, 2% FBS, 50nM testosterone, 10ng/ml EGF, 10ng/ml cholera toxin, 500nM dexamethasone, 5μg/ml insulin, 25μg/ml BPE, penicillin, and streptomycin. In addition to selective medium, explants were plated on Vitrogen collagen-coated Millicell-CM culture inserts to select for epithelial outgrowth. To further purify the epithelial cultures, trypsinized cells were allowed to plate overnight on polystyrene dishes in YD medium. Unattached cells were then transferred to Millicell-CM inserts, which resulted in pure epithelial outgrowth. Carcinoma-derived stromal cells were cultured in the same way as normal cells, while tumor epithelium plated and grew well in YD medium on collagen-coated polystyrene dishes. As the cultures progressed, cell types were identified by morphological appearances and immunohistochemical staining for cytokeratin and vimentin. Once pure cultures were established, cells were characterized in terms of their doubling times, androgen responsiveness, and karyotype.

V-1006 Nitric Oxide Production in Lymphatic Endothelial Cells In Vitro. L.V.Leak. Ernest E. Just Laboratory of Cellular Biology, Department of Anatomy, College of Medicine, Howard University, Washington, DC 20059.

To examine whether nitric oxide (NO) is involved as a lymphatic endothelial-derived relaxing factor, NO production by the lymphatic endothelium was quantitated by the colorimetric Griess reaction for NO_2 in culture medium conditioned by confluent monolayers of LEC. Basal levels of NO production by confluent LEC monolayers was 65 uM of NO_2 for 10^6 cells/24 hrs. Immunofluorescence studies showed an enhanced eNOS activity with Ca ionophore in monolayers and in lymphangiogenesis. In addition, treatment with LPS, histamine and various cytokines also stimulated the production of iNOS which showed the greatest increase in activity at 4 hrs and declined at 18 hrs. The results show a dose dependent response with histamine producing the greatest response (120 uM/10^6 cells/48 hrs. at 1mM) compared with LPS (98 uM/10^6 cell/48 hrs. at 100 nM). The iNO production was significantly reduced with the NO synthase inhibitor N-nitroarginine methyl ester at 500 uM. These results suggest a possible involvement of NO in the vasodilation of lymphatic vessels in regulating the lymphatic vascular tone for lymph propulsion through lymph vessels and lymph nodes.

V-1007 Longterm alteration in the expression of cell cycle control and signal transduction genes following exposure of human urothelial cultures to gamma radiation. CARMEL MOTHERSILL, J. Harney, F. Lyng, C. Seymour, K. Parsons and D. Murphy. Dublin Institute of Technology, Kevin St., Dublin, and Depts of Urology, Beaumont Hospital, Dublin, and Royal Hospital, Liverpool.

Urothelium from normal patients was cultured in Clonetics KGM (San Diego Ca) using an explant technique. The explants were irradiated in the range 0.1-5 Gy using cobalt 60 gamma rays on day 2 of culture. They were allowed to grow for up to 30 days post irradiation. At various time points cultures were fixed and stained to demonstrate the status of the following oncoproteins: $p53^{mut}$, cmyc, bcl-2, $p21^{ras}$, EGFr and mdm2. The mean results for over 100 patients without any form of tumour, showed significant (ranging from $p<0.05$-0.001) induction of all these proteins following doses as low as 0.1Gy (10rads). The effects showed a time course with great variations in oncoprotein levels in the first 4 hours after irradiation but these stabilised after this time to give prolonged, stable and singificant increases. When the results for individual patients were analysed significant patient variation was seen, particularly for $p53^{mut}$ induction where30% of patients showed no induction of the stable oncoprotein at any radiation doses or time point tested. The results are interpreted to suggest a general deregulation in growth control and signal transduction following irradiation even to very low doses. Given the current interest in genetic instability as a longterm consequence of radiation exposure the results may provide a mechanism by which such instability could come about.

V-1008 Effects of Vitamin D and its Analogs on Breast Carcinoma Cells. MEHTA, R.R., Mehta, R.G., Das Gupta, T.K. Dept. of Surgical Oncology, Univ. of Illinois, Chicago, IL 60612.

Vitamin D and its analogs modulate proliferation and induce differentiation in malignant cells, but how they inhibit growth is still unknown. Using UISO-BCA-1 and UISO-BCA-4 human breast carcinoma cell lines, we examined the effects of 4 vitamin D analogs: $1\alpha,25(OH)_2D_3$ (D_3), $1\alpha,(OH)D_3$ ($1\alpha D_3$), 1α hydroxy 16-ene-26,27 hexafluoro D_3 (R024-5531), and 1α hydroxy 25 ethyl D_3 (D5). All analogs tested showed dose-related growth inhibitory action in BCA-4 cells only. After 10 days exposure at 100 nM concentration, D_3 had 81%, $1\alpha D_3$ 60%, D5 30%, and R024-5531 61% growth inhibition, compared to untreated cells. After vitamin treatment, typical morphologic changes (fragmented nuclei, numerous vacuoles, thin cytoplasm) were observed in BCA-4. Increased expression of differentiation marker ICAM-I (CD 54) was also observed in treated cells. Treated BCA-4 cells, but not BCA-1, showed increased expression of intracellular $TGF\beta_1$ and vitamin D receptors (VDR) compared to untreated cells. Relative induction of $TGF\beta_1$ in BCA-4 cells correlated with the antiproliferative potency of analogs. Our data suggest that cell differentiation and inhibition of growth by vitamin D and its analogs are mediated via VDR and that induction of $TGF\beta_1$ may be a common mechanism for their actions.

V-1009 Experimental Down-Regulation of c-myc Oncogene-Induced Transformation In Mammary Epithelial Cells: Effect of Brassinin Derivatives: N.T. TELANG, S. Inoue, R.G. Mehta, R.M. Moriarty, H.L. Bradlow, M.P. Osborne. Strang-Cornell Cancer Research Laboratory. Cornell University Medical College, New York; Depts of Surgical Oncology and Chemistry, University of Illinois, Chicago, Illinois.

Deregulated expression of the c-myc oncogene confers neoplastic transformation in mammary epithelial cells [Telang etal Cell Regulation 1:863-872,1990]. The stable myc transfecant MMEC/myc_3 exhibits aberrant hyperproliferation in vitro and hyperplasia in vivo prior to tumorigenesis. Present experiments were designed to examine whether selected derivatives of Brassinin, a naturally-occurring phytoalexin from cruciferous plants, alter oncogene-induced cell transformation in MMEC/myc_3 cells. The transformation markers included i.) aberrant hyperproliferation as measured by anchorage-dependent growth (ADG) and anchorage-independent growth (AIG), and ii.) cellular metabolism of estradiol (E_2). A continuous 96hr. treatment of MMEC/myc_3 cells with 50uM indole-3-carbinol (I3C), Brassinin (BRS), cyclobrassinin (c-BRS) and 4-methylbrassinin (4-Me BRS) resulted in a growth inhibition range of 32.6%-94.1% relative to that with 0.1% DMSO (solvent control). [4-MeBRS > BRS > c-BRS > I3C > DMSO]. At the nontoxic doses of 10uM (growth inhibition: <10%), the test compounds exhibited an AIG inhibition range of 21.3%-57.6% with a corresponding increase of E_2 C2-hydroxylation leading to the formation of antiproliferative 2-hydroxyestrone (2-OHE_1). Thus, naturally-occurring phytoalexins may inhibit myc oncogene induced mammary cell transformation in part by upregulating C2-hydroxylation of E_2. [Support: P01 CA 29502, P01 CA 48112 and philanthropic funds to the Strang-Cancer Prevention Center].

V-1010 Human Virus Detection using Cells Immortalised by Oncogenes. J B CLARKE, H Moulsdale, J Golding, B Griffiths: CAMR, Porton Down, Salisbury, SP4 0JG, UK; P Chakraverty: CPHL, Colindale, London, UK; U Kreuzberg-Duffy and C MacDonald: University of Paisley, Paisley, UK.

Diagnostic laboratories in the UK use primary monkey kidney (PMK) cells as their main substrate for virus detection. Unfortunately, these have only limited subculture capability, and therefore both ethical and practical considerations impel a search for alternatives. Direct techniques, such as immunofluorescent staining, nucleic acid hybridisation and PCR, require virus-specific probes and would fail to detect an unsuspected virus or multiple infection. Attempts have therefore been made to derive a cell-based system to replace PMK cells. No single existing established cell line was found that could by itself substitute for PMK cells. A panel of cell lines could detect the more important viruses requiring routine diagnosis, but less sensitively than PMK. In a promising alternative approach, oncogene transfection was used to establish thirty immortalised baboon kidney and thirty Rhesus macaque kidney cell lines. Some of the baboon lines supported the test viruses, but not so well as primary baboon kidney cell cultures. However, five of the Rhesus kidney lines appear to have similar virus susceptibility to their parent primary cells and are being further evaluated.

V-1011 The Development of a Model of Cancer Initiation and Progression using Conditionally Immortalised Colonic Mucosal Cells. R.H. WHITEHEAD, J. Weinstock ,J.L. Joseph. Ludwig Institute for Cancer Research, P.O. Royal Melbourne Hospital, Melbourne, 3050, AUSTRALIA

Studies on the steps leading to oncogenesis in colonic mucosal cells have been hampered by our inability to routinely culture normal intestinal mucosal cells *in vitro*. We have described the culture of colonic mucosal cells from adult mice using an unique transgenic mouse containing a ts mutant of the SV40 large T gene (Immortomouse). The availability of this mouse has allowed us to develop cell lines containing genetic mutations shown to be present in colon cancer by Vogelstein's group and others. Initially, a heterozygous Min mouse (murine APC mutant) and a heterozygous 'Immortomouse' were mated and the litter tested for the presence of both the SV40 large T gene and the Min gene by PCR. Tissues were removed from mice expressing both genes at 5 weeks of age and cultured. Conditionally immortalised cell lines of colonic, small intestinal and liver epithelium and intestinal stromal fibroblasts have been obtained. All 4 cell lines contain both the SV40 large T and the Min genes. Similarly a Min/Immortomouse cross has been mated with an 'Oncomouse' (H-Ras mutation) and a cell line derived from the colonic mucosa. This cell line contains all 3 mutations. Initial results indicate that the presence of one mutant Min gene in these cells does not affect the phenotype of the cells as the cells still demonstrate temperature-sensitive growth and do not clone in soft agar or form tumours in nude mice. Studies on the phenotype of the Min/Immortomouse/Ras cell lines are in progress. When a DCC knockout mouse becomes available we should be able to develop colonic cell lines that contain all of the mutations present in the Vogelstein schema.

V-1012 A New Culture Method of Three-Dimensionally Reconstituted Multicellular Mass Utilizing Cotton-Gauze. T. TAKEZAWA* and K. Yoshizato. Yoshizato MorphoMatrix Project, ERATO, JRDC, Hiroshima-TechnoPlaza, 242-37 Saijo-cho Misonou, HigashiHiroshima, 724 Japan. *Present address: Corporate research Division, Japan Technical Center, Procter & Gamble Far East, Inc., 10F-HM, 1-17 Koyo-cho Naka, Higashinada-ku, Kobe, 658 Japan.

There have been no trials to incorporate vascular capillaries into the conventional cell culture system of three-dimensionally reconstituted multicellular masses (3-DRMMs) such as multicellular spheroids and cell-embedded collagen gels. In the present study, fibroblasts were cultured on collagen-coated fibers of cotton-gauze that had been attached to culture dishes and were allowed to become confluent. A multilayer sheet of cells was detached from the dish and cultured for 21 days in the apparatus in which medium was circulating by a flow rate controlled by a peristaltic pump. Pyknotic and necrotic cells were histologically observed in spheroids with a diameter of 600μm which had been prepared and cultured by a conventional method[1] without mesh works and circulation. In contrast, no such necrotic cells were seen in the 3-DRMM with mesh works which was cultured in circulating medium, in spite of the fact that its size was larger than the spheroid. Electron microscopic survey revealed the presence of collagen fibrils between cells in the mesh work-containing 3-DRMM, but not in the conventional 3-DRMM. 1) T. Takezawa et al., Exp. Cell Res., 208, 430-441, 1993.

V-1013

Archival Storage and Analytical Display of Culture Media. E.K. WHITE, T. Cuffel and R.G. Ham, M.C.D. Biology, CB 347, Univ. of Colorado, Boulder, CO 80309.

Systematic storage and analytical comparisons of cell culture media formulations are difficult because of their large and variable number of components, diverse chemical forms of the components, and use of either weight or molar concentrations. We are developing a computerized system that minimizes these difficulties. Data are entered using names and units from the original publications. A file of synonyms is used to link each entry to a standard nomenclature, to identify interchangability (e.g. pyridoxal = pyridoxine), and to assign a molecular weight, an osmotic multiplier, and multipliers for summation of shared ions such as sodium, potassium, and chloride. Molar and weight concentrations are calculated as needed, permitting display of both. For quantitative comparisons, ratios of components (or nutritional equivalents) in two media can be displayed in sequence from lowest to highest values, including zero and infinity if each contains items not in the other. A graphic display has been designed to visualize small differences among media in relative and absolute levels of common nutrients. Components are clustered by type and displayed on a log molar scale as bar graphs extending up or down from "typical" levels for such components. For example, amino acids are displayed as bars up or down from 1.0E-4 M. A low amino acid medium, such as F10, has many bars extending downward, whereas one with high levels, such as L15, has many bars extending upward. Vitamins are displayed around 1.0E-6 M, other organics around 1.0E-5 M, bulk ions around 1.0E-3 M, and trace elements around 1.0E-8 M. Within each group, the components are arranged to facilitate visual detection of differences.

V-1014

Viral Inactivation of Serum with Ultra Violet (UV) Irradiation. V. H. WILLIAMSON, PAA Laboratories, Inc. Newport Beach, CA 92660.

PAA Laboratories, Inc., headquartered in Linz, Austria, has developed a proprietory system 'Viralex' tm utilizing exposure of serum in a thin layer to UV irradiation, for a predetermined time, during the sterile filtration process.

The method has been optimized to preserve the growth promoting integrity of the serum, while maximizing the log viral inactivation. Models chosen for this validation study with fetal bovine serum, by Microbiological Associates, are bovine viral diarrhea virus (BVD), bovine parvovirus (BPV), parainfluenza virus type III (PI-3), infectious rhinotracheitis virus (IBR), reovirus type 3 (REO) and acholeplasma laidlaw II.

Greater than 8 logs inactivation is achieved for BVD and BPV, greater than 7 logs for PI-3 and acholeplasma laidlaw II with greater than 4 logs for IBR and REO.

V-1015

The Effect of Platinum Pharmacokinetics on the Growth of Low-Dose-Rate Radiation Resistant Peripheral Blood Lymphocytes. S.L. SCHNEIDER, M. Szekeresova, and M. DeGregorio. School of Public Health at Houston and Department of Medicine/Oncology, The University of Texas Health Science Center, San Antonio, TX 78284-7976.

Cisplatin (*cis*-platinum-diammine-dichloride) functions as an alkylating agent. This agent has been used in combination with low-dose-rate irradiation in the treatment of various cancers including head and neck, cervical, lung, and ovarian cancers. The mechanism for cisplatin enhanced radiation effects is not fully understood. In the present study, the lymphoid cell line 244B, obtained from Epstein Barr Virus (EBV)-transformed normal, human peripheral blood cells, was used to determine the effects of *cis*platin on radiation resistant lymphocytes. 244B cells selected for resistance to ionizing radiation at 100cGy and 500cGy were exposed to 0 to 10 ug/ml of cisplatin for 96 hours, and compared to radiation sensitive control 244B cells. Inhibition of cellular proliferation was determined by cell kinetics and viability, and reported as percent of control survival. Cellular dose-response survival of the radiation sensitive 244B cell line to 1ug, 5ug, and 10 ug cisplatin was 84.5%, 26.8%, and 10.2% respectively. The survival response of 100cGy resistant 244B cells was 100%, 57.4% and 40.4% respectively, while the survival response of 500cGy resistant 244B cells was 100%, 69.6% and 62.6% respectively. The subsequent 100cGy low-dose-rate irradiation of the radioresistant cells treated with cisplatin resulted in enhanced cellular destruction, similar to that observed in clinical studies. The 244B cell line is a useful *in vitro* model to study the effects of multi-drug resistance and combinations of low-dose-rate irradiation in cells of the immune system.

V-1016

Abstract has been withdrawn.

V-1017

Melatonin modulates the inhibitory action of some chlorinated acids on intercellular communication. S. G. BENANE, C. F. Blackman, D. E. House. US EPA, Reasearch Triangle Park, NC 27711

When chlorine is used to purify drinking water, it reacts with organic matter in raw water forming chloracetic acids and their correspond-ing chloroaldehydes. These by-products have been shown to affect intercellular communication (IC) with different efficiencies in cultured cells. Melatonin, a prominent naturally occurring hormone, is known to have oncostatic properties. We have recently reported that physiological concentrations of melatonin can enhance IC in 10T1/2 cells. We now ask if melatonin can modulate the inhibition of IC caused by these water purification by-products? Here we examined that potential using Clone 9 cell cultures (normal rat liver cells). In this study we selected concentrations of chemicals that had been previously shown to reduce IC by approximately one third compared to controls. The chemicals and the concentrations chosen are: dichloroacetic acid (DCA) at 10 mM and 20 mM, trichloroacetic acid (TCA) at 2.5 mM, chloral hydrate (CH) at 5 mM, trichloroethylene (TCEth) at 5 mM, and perchloroethylene (PERC) at 0.1mM. Lucifer Yellow scrape-load dye transfer was used to assay for IC. The doses of melatonin used are in the physiological range: 1, 2.5, 5, 10, 20, 40 and 80 $x10^{-10}$ M. Melatonin had no effect on IC in Clone 9 cells not exposed to a chemical, nor when the cells were exposed to TCEth or PERC. Exposure to DCA at 10 mM and CH at 5 mM, caused an increase in IC at a melatonin concentration of 40 $x10^{-10}$ M, with a maximum increase at 20 $x10^{-10}$. Cells exposed to DCA at 20 mM demonstrated increased IC at melatonin concentrations of 10 and 20 $x10^{-10}$ M. IC in cells treated with TCA showed increased IC at melatonin concentrations of 2.5 $x10^{-10}$ and at 10 $x10^{-10}$ M with a maximum increase at 5 $x10^{-10}$ M. These results demonstrate for the first time that physiological concentrations of melatonin can modulate the inhibition of IC caused by some of the water purification by-products or metabolites. This modulation may be the basis for some of the oncostatic properties attributed to melatonin. Additional work is necessary to establish the mechanism for this process and to determine if it operates *in vivo*.

V-1018

Isolation and Characterization of Monoclonal Antibodies to Surface Molecules of a Compaction-Defective Mutant of the Multicell Tumor Spheroid Phenotype. S.N. GARCIA, P. Pineda, L.A. Jordan, L.S. Armstrong and A.O. Martinez. Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78249.

Multicell tumor spheroids (MTS) are being used in our laboratory as *in vitro* models to study cell-cell interactions of tumor and transformed cells. MTS are formed via a two-step process involving cell aggregation and compaction, mediated by cell-cell interactions. We have isolated several lectin-resistant mutants of B14I50 (hamster) cells which are defective in expression of the MTS phenotype. In this study, we have used a subtractive immunization technique to generate monoclonal antibodies (MAbs) directed against surface molecules of a B14I50 mutant— ConAR-2DF-F1 (2DF)— defective in compaction. Screening of MAbs by immunofluorescence microscopy revealed two major types of surface-binding MAbs: One type preferentially bound to the intercellular boundaries of both 2DF and B14I50 cells grown as confluent monolayers. A second type bound to both the intercellular boundaries and extracellular matrix of 2DF cells only. Further characterization of these MAbs by flow cytometry showed that the first type bound strongly to both B14I50 and 2DF single cells. In contrast, the second type bound strongly to 2DF single cells but failed to bind to B14I50 single cells. These MAbs may be important in identifying and characterizing surface molecules involved in the cell-cell interactions leading to MTS (and perhaps tumor) formation. (Supported by NIH (GM08194, GM07717), NSF (RII-9005546) and NASA (NAG-2-819) grants.)

V-1019

Analysis of Somatic Cell Hybrids Between MTS^+ and MTS^- Cell Lines for Expression of Multicell Tumor Spheroid (MTS^+) Phenotype. M.L. UBINAS, J. Martinez, J. Pizarro, L.S. Armstrong and A.O. Martinez. Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78249.

In our laboratory we are using multicell tumor spheroids (MTS) as models to study genetic mechanisms involved in cell-cell interactions of tumor and transformed cells. MTS are highly organized 3-dimensional *in vitro* structures which offer many characteristics of *in vivo* tumors, including intimate cell-cell contacts and tumor microenvironments. They are formed via a two-step process mediated by cell-cell interactions. In the first step, single cells in suspension interact to form loosely adherent "cell aggregates". In the second step, cell aggregates compact into smooth, organized multicellular structures capable of 3-dimensional growth. For this study, we have generated and characterized a number of interspecific somatic cell hybrids between MTS^+ and MTS^- cell lines in order to study the genetic mechanism(s) involved in expression of the MTS phenotype. Hybrids were produced by PEG-induced somatic cell fusion and their hybrid nature verified by isoenzyme analysis. Two general hybrid phenotypes were observed: One phenotype exhibited the ability to both aggregate and compact into MTS. A second phenotype exhibited the ability to aggregate but not to compact into MTS. The results obtained appear to indicate that MTS formation— i.e., aggregation and compaction— is controlled by different genetic mechanisms, and support the hypothesis that multiple genes are involved in expression of the MTS phenotype. We are currently generating a hybrid-cell panel to map MTS genes to specific human chromosome(s).
(Supported by NIH (GM08194, GM07717), NSF (RII-9005546) and NASA (NAG-2-819) grants.)

V-1020

A New Monoclonal Antibody 5G7 Reacting with Human Leukocytes. E.DIMITROVA, H.Taskov, M.Nikolova, A.Pashov. Laboratory of Monoclonal Antibodies, National Centre of Infectious and Parasitic Diseases, Sofia 1504, Bulgaria.

A new murine hybridoma 5G7 was raised using as antigen human leukocytes, isolated from peripheral blood of healthy donors by gradient centrifugation on Ficoll-Paque. It produced monoclonal antibody reacting with human leukocytes. The antigen was strongly expressed on granulocytes and monocytes and weakly on lymphocytes. It was not expressed on erythrocytes and platelets. The cell lines Jurkat, CEM, MOLT4, MONOMAC6, Raji, Reh and K562 were positive for 5G7.
5G7 immunoprecipitated an antigen which subjected to SDS-PAGE under reducing and nonreducing conditions migrated as a single band with MW 46-50 kD.
5G7 was found strongly expressed on leukaemic cells and on cells, activated by PHA, SAC and LPS.

V-1021

Human Hydatidiform Mole in Culture : A Multi-Nucleated Trophoblast Cell Line. DON THOMPSON, Gloria E.Sarto MD. Ph.D., Maxine Dorin M.D. , University of New Mexico, Health Science Center, Dept. OB/GYN. Albuquerque, New Mexico 87131.

Successful growth of trophoblast in vitro from hydatidiform mole (HM) is dependent upon the method of collection , storage before processing, growth media, and incubation. The following is a description of a method used to establish cultures in our laboratory. Two primary in vitro cultures were established using HM tissue from separate surgical terminations. The tissue was removed from the uterus with a ring forceps under sterile conditions. Since the specimens were obtained at odd hours the tissue was placed in DMEM with gentamycin and kept at 1-2° C for up to 15 hours before processing. Only tissue forming the "grape - like" structure was used. All clotted blood was removed; the remaining tissue was washed 2x in HBSS with gentamycin. The tissue was cut into 1-2 mm squares, placed into a digestion flask with a trypsin solution and was stirred at a low speed for 30 minutes at 37° C. The HM cells were placed in KDM (Clonetics) media /20% FBS and plated into a 25 cm^2 tissue culture flask (Corning) with vented membrane cap and incubated at 37° C in 5% CO_2. The KDM media was replaced after 48 hours to remove any nonviable tissue pieces and RBC's. Immunohistochemical staining showed strong activity for human chorionic gonadotropin (hCG), vimentin and alpha actin. Many of the cells showed extreme mobility; actin fibers were visible under phase contrast. In summary, HM tissue in vitro may serve as a possible model to investigate autocrine / endocrine regulation in trophoblast growth and differentiation associated with gestational trophoblastic disease.

V-1022

Growth Factors Produced by the LA7 Rat Mammary Tumor Cell Line Stimulate Proliferation of Mouse Mammary Epithelial Cells. U.K. EHMANN, J.T. De Vries, M.S.C. Chen, and A.A. Adamos. Pathology and Laboratory Service 151B, Veterans Affairs Medical Center, 3801 Miranda Ave., Palo Alto, CA 94304.

When epithelial cells from mouse mammary glands are placed in culture with cells of the rat mammary LA7 tumor line they proliferate extensively. Contact between the two cell types has been shown to be necessary for growth stimulation, but the exact nature of the growth stimulants has not been known. Because growth stimulating activity has been demonstrated in concentrated LA7-conditioned medium, we have screened it for several growth factors. Three growth factors, namely, transforming growth factor α, basic fibroblast growth factor, and int-2, have been identified in LA7-conditioned media as well as LA7 cell lysates. Their molecular weights were confirmed by polyacrylamide gel electrophoresis followed by antibody probing of Western blots. These growth factors have also been detected on fixed, cultured LA7 cells by immunocytochemistry. At the present time we are assessing the importance of these factors in growth stimulation of mouse mammary epithelial cells.

V-1023

Caloric Restriction In Vitro: Role of Serum on Cultured Adipocytes from Rats Fed Ad Libitum (AL) and Calorically Restricted (CR) Diets. BRUCE S. HASS, R.W. Hart, Neil A. Littlefield, A.Turturro. National Center for Toxicological Research, Jefferson AR 72079.

A hallmark of caloric restriction is the slower growth of cells from animals fed CR diets.This tendency toward slower growth persists for 15 passages after cells have been excised from animals (*Carcinogenesis* 13: 2419 [1992]).A critical question concerns the cause of the slower growth, which could be due to the paucity of energy sources (glucose, glutamine) or to non-energy factors that control the rate of growth (hormones like insulin).We have cultured fat cells from F344-BNF1 rats fed AL or CR diets in characterized fetal bovine serum (FBS) or bovine calf serum (BCS) to determine if the additional hormonal constituents found in BCS might give a clue to the differential in growth characteristics. In terms of the population doubling level: AL_{BCS} (2.47) ~ AL_{FBS}(2.64); CR_{BCS} (1.53) ~ CR_{FBS}(1.53). Obviously AL_{BCS}>CR_{BCS} and AL_{FBS}>CR_{FBS}, supporting our previous finding that cells from AL animals grow faster than cells from CR animals. However, since FBS induces a higher plating efficiency (~2x) in both AL and CR cells, a lower specific growth rate for cells in the hormonally-deficient FBS is indicated while a higher cell population is maintained. Cell size increases in AL_{BCS}, CR_{BCS}, and CR_{FBS} cultures implying a trend toward a more differentiated (slower growing) state than in AL_{FBS} cells which remain small and have a higher proliferation rate and plating efficiency. Thus, the more hormone-laden BCS promotes the faster growth of cells from AL animals and slower growth in cells from CR animals, a result that mimics the in vivo response of cells under AL and CR diets. These results do not rule out energy sources as a factor in the CR response of individual cells, but they do implicate hormonal control as playing a possible role in the CR response. The adipocyte system cultured in BCS may define a hormone-sensitive model for mimicking CR in vitro.

V-1024

Transport Mechanism of Histamine Receptor Type 2 (H_2) Antagonists in Caco-2 cells. H.H. FARRISH, S.B. Yanni L-S. Gan and P-H. Hsyu,. Glaxo Research Institute, Research Triangle Park, NC 27709.

H_2 antagonists are currently the most important drugs for the treatment of gastric and intestinal ulcers. The marketed H_2 antagonists cimetidine, ranitidine, famotidine, and nizatidine have different bioavailability values ranging from 40 to 90%. In this study, we investigated the absorption mechanism of these H_2 antagonists in the Caco-2 cells, an in vitro model for drug absorption in human intestine. We studied the transport rate of these drugs at various concentrations. Apparent permeability (Papp) of each drug was determined. Calcium switching assay with EDTA was used to further delineate the transport route of these drugs. Relationship between the transport rate and the concentration was linear for each compound suggesting that passive diffusion is the predominant process of transport in the Caco-2 cells. Papp values of cimetidine, ranitidine, famotidine, and nizatidine were 2.93, 2.20, 1.84, and 5.86 (10^{-7} cm/s), respectively. The rank order of Papp values correspond to the rank order of bioavailability values of these compounds suggesting that intestinal permeability might be a major factor for oral bioavailability. In the presence of 10 mM EDTA, which chelated with calcium ion and opened up the tight junctions, the Papp value of cimetidine, ranitidine, and famotidine were increased to 200, 330, 200%, respectively; whereas the Papp value of nizatidine was not affected. These results suggested that nizatidine was predominantly transported by the transcelluar route and the other H_2 antagonists were predominantly transported by the paracellular route.

V-1025

Serum-free Media for Growth of Seven Mammalian Kidney Cell Types. J.A. DARNER, P. Miller, F. Simon and B.A. Van der Haegen. BioWhittaker, Inc., Walkersville, MD 21793.

In recent years it has become evident that, due to increasing safety concerns, the ability to grow cells in serum-free media has increased in importance. We have developed serum-free media for seven kidney cell lines widely used in virology and vaccine production. "UltraMDCK" is a defined, optimized serum-free medium designed to support growth of MDCK (Madin Darby Canine Kidney) cells. This medium is supplemented with only two proteins, insulin and transferrin, yielding a total protein concentration of 20 µg/ml. "Nephros" is a serum-free medium formulated to support growth of African Green Monkey Kidney and Vero cells as well as Rhesus Monkey Kidney cells. The "Nephros" formulation has a total protein concentration of 205 µg/ml and does not include any animal-derived protein. "Nephros LP" is a low protein, serum-free medium optimized for growth of CRFK (feline kidney cells), PK-15 (porcine kidney cells) and BGMK (Buffalo Green Monkey kidney cells). "Nephros LP" is supplemented with recombinant growth factors and has a total protein concentration of 10 µg/ml. UltraMDCK, NEPHROS, and NEPHROS LP are designed as growth medium for expansion of stock cultures and do *not* require weaning procedures. Their use during viral infection requires monitoring since medium composition may affect infectivity. "UltraMDCK" enhances viral infection with several types of viruses. When these serum-free media are used in roller cultures, bottle rotational speed should be set around 20 rev/hr to allow cells to attach. Maximum speed should not exceed 50 rev/hr. Because cells cultured in serum-free media are very sensitive to proteases, trypsinization procedures must be performed carefully. In our hands, the use of a cold trypsinization protocol substantially improves cell yield in serum-free culture systems.

V-1026

Characterization of Skeletal Muscle Atrophy Induced in Simulated Microgravity Culture Systems. D. BROWN*, K.I. Clark*, N.R. Pellis**, and T.J. Goodwin**. *U. Michigan, Ann Arbor, MI 48109 and **KRUG Life Sciences and **NASA-Johnson Space Center, Houston, TX 77058

Clinical data on recovery from injuries and examinations of astronauts upon returning from space flight demonstrate significant atrophy of skeletal muscle tissue as a result of relatively brief periods of disuse. In vitro models of microgravity-induced human and murine skeletal muscle degeneration have been developed using the Johnson Space Center designed rotating wall vessel, a low shear stress, three dimensional tissue culture bioreactor that simulates microgravity conditions. We used the SKMC human striated skeletal muscle cell line (Clonetics, San Diego, CA) and the C_2C_{12} murine skeletal muscle cell line (ATCC, Rockville, MD). Both cell lines demonstrate normal muscle physiology upon differentiation. Undifferentiated cells were seeded into the bioreactor along with collagen coated microcarrier beads (Sigma, St. Louis, Mo.) at a concentration of $2.0x10^5$cells/ml. In addition, cells were seeded into 25 cm^2 flasks alone and into Petri dishes with microcarrier beads as a 1 gravity (g) control. Upon differentiation, the 1g control cells exhibited the characteristics of mature muscle tissue, such as development of myotubules, multinucleation, elongation, and production of creatine kinase in both cell lines. Conversely, the bioreactor cell cultures degenerated and exhibited none of the traits of mature muscle. These data support the application of the bioreactor culture system as an *in vitro* model for examining the atrophic effects of muscle disuse. This work is supported by NASA- Johnson Space Center and Ames research grant NCC2-856.

V-1027

Antagonistic Actions of Triiodothyronine and Dexamethasone on the Differentiation of Cultured Adult Human Jaw Bone Osteoblasts. C. Guerriero[1], D. De Santis[2], P. Gotte[2], P.F. Nocini[2], and U. ARMATO[1] [1] Institute of Anatomy and Histology, and [2] Dental Department, University of Verona, Verona, Italy

The endocrine mechanisms that control the expression of differentiated features in human osteoblasts are only partially understood; moreover, they might vary from place to place. The present work explored the interactions between triiodothyronine (T_3) and dexamethasone (Dex) on the functioning of normal adult human osteoblasts. These cells outgrew from mandibular or maxillary bone fragments predigested with collagenase and were further expanded in low-Ca^{2+} (0.2 m*M*)DMEM medium fortified with FBS (10% v/v). At the onset of the experiments, the bone cells were shifted to a ß-glycerophosphate (10 m*M*)- and ascorbate (50 µg/ml)-enriched high-Ca^{2+} (1.8 mM) DMEM medium. Part of the specimens was treated with an equimolar (10^{-8} *M*) mixture of A and D vitamins and Dex (A+D+Dex). Other cultures either received plain fresh medium or were incubated with the A+D+Dex mixture with T_3 (2×10^{-9} *M*) added. The exposure to the A+D+Dex combination significantly increased, in comparison with the parallel controls, the cellular alkaline phosphatase (ALP) activity between day 5 and day 15 of staying in culture. Besides, a remarkable deposition of calcium phosphate salts could be observed in these same specimens. On the other hand, the administration, between the 5th and 15th day, of T_3 together with A and D vitamins also increased, though to a lesser degree, the cell ALP activity. Conversely, the simultaneous administration of T_3 and the A+D+Dex mixture prevented any significant increase in ALP activity from occurring. These results show that A and D vitamins and Dex synergistically favor the expression of the adult human osteoblastic phenotype. They also show that this action of Dex can be effectively counteracted by the presence of T_3. These findings increase our understanding of the biology and pathology of the human bone.

V-1028

In Vitro Production of Basement Membrane Extracellular Matrix by Human Umbilical Venous Endothelial Cells. E.J. ROEMER, M. Spektor, and S.R. Simon. Department of Pathology, SUNY at Stony Brook, Stony Brook NY 11794-8691

We have previously developed a method for producing interstitial extracellular matrix (ECM) for use in *in vitro* assays and are now exploring the extension of this technology to basement membrane ECM (BMECM). Human umbilical venous endothelial cells (HUVEC) were grown in three commercial media: Gibco/BRL serum-free endothelial cell medium, Clonetics Endothelial Growth Medium (containing 2% FBS), and B-D/Collaborative Biomedical Products medium (also containing 2% FBS). Cells were plated on either gelatin or R22 rat smooth muscle cell-derived interstitial ECM in flasks or multiwell plates, grown to confluence and maintained in postconfluent culture for 1-3 weeks. Radiolabeled precursors were added to the growth media to assess quantitatively the production of metabolically labeled ECM components and cultures were supplemented with ascorbic acid to support formation of an insoluble crosslinked matrix. Growing cultures were rountinely examined by phase contrast microscopy and were fixed at various time points for further morphological evaluation by light microscopy. For biochemical analysis of BMECM, HUVEC were lysed with 25 mM NH_4OH and the insoluble matrix remaining in the multiwell plates was subjected to sequential digestion with trypsin, collagenase, and elastase. Preliminary data indicates that HUVEC grown on an interstitial ECM substrate in serum-free medium incorporate radiolabeled alanine, proline, fucose and sulfate into an extracellular material which is insoluble in 25 mM NH_4OH or repeated washes with PBS but which is degraded by sequential treatment with trypsin and collagenase. (Supported by NIH [DE-10985], Smokeless Tobacco Research Council, SUSB Biotechnology Center, Collaborative Laboratories, and Chiroscience, Ltd.)

V-1029

An Improved Method for Ascorbate Supplementation of R22 Cells in Culture for *In Vitro* Biosynthesis of Interstitial Extracellular Matrix. E.J. ROEMER, M. Spektor, and S.R. Simon. Department of Pathology, SUNY Stony Brook, Stony Brook NY 11794-8691

We have adapted protocols for the production of R22 rat smooth muscle cell-derived interstitial extracellular matrix (ECM) on polystyrene multiwell plates and Cell Culture membrane inserts with a range of porosities. R22 cells were plated onto multiwell tissue culture treated polystyrene plates or cell culture inserts at a density of 2.5 x 10^4 cells/cm^2 in Modified Eagle's Medium (MEM) supplemented with fetal bovine serum (FBS), tryptose phosphate broth, and antibiotic. All the medium was removed every four days and replaced with fresh medium. For selective radiolabeling of ECM components, cells were grown in medium containing one or more of the following precursors: [^{35}S]-methionine/cysteine TranS Label, [^{35}S] sulfate, [^{3}H] fucose, [^{3}H] proline or [^{14}C] proline. Previously, confluent cultures were supplemented with 50 µg/ml ascorbic acid daily for 8 - 10 days until sufficient insoluble crosslinked ECM had formed for use. We have replaced the labile free acid with l-ascorbic acid phosphate magnesium salt, which is stable in culture. Data indicate that addition of 50 µg/ml of this salt to the medium which is replaced every four days supports biosynthesis of insoluble ECM at rates similar to those achieved with daily supplementation with free ascorbic acid. Analysis by sequential digestion of ECM elaborated by cells in culture with ascorbate phosphate demonstrates that matrix components are produced in the same ratios as in cultures receiving daily supplementation with free ascorbate. The use of a non-labile form of ascorbate greatly simplifies ECM production and significantly decreases the frequency of handling that the cultures require, thereby reducing risk of contamination as well as saving time and money. (Supported by NIH [DE-10985], Smokeless Tobacco Research Council, SUSB Biotechnology Center, Collaborative Laboratories, and Chiroscience, Ltd.)

V-1030

Divalent Cations and Assembly of the FGF Receptor Complex. M. KAN, F. Wang and W.L. McKeehan. Center for Cancer Biology, Institute of Biosciences and Technology, Texas A&M University, Houston, TX 77030.

We have proposed that the fibroblast growth factor receptor (FGFR) is a ternary complex of FGF, the ectodomain of the transmembrane tyrosine kinase and heparan sulfate, each of which has distinct binding domains for the other. Conflicting reports in the literature suggest that heparin, which mimics native cell surface heparan sulfates, is obligatory for FGF binding and activity, increases affinity of FGF for the receptor or has no effect on FGF receptor binding. We have investigated conditions for ligand binding to recombinant receptor expressed in baculoviral-infected Sf9 insect cells as well as the purified receptor extracted from the cells followed by immobilization on agarose beads. At or greater than physiological levels of the divalent cations Mg2+, Ca2+, or Mn2+, high-affinity receptor complex formation with radiolabeled FGF was heparin-dependent. In the absence of divalent ions, FGF bound to the recombinant ectodomain of the kinase in a conformation that could be covalently cross-linked to it in the absence of heparin. However, the addition of heparin increased the affinity of FGF to the complex. Although the independent interaction of FGF and heparin was not affected by divalent ions, the independent interaction of receptor kinase ectodomain and heparin was dependent on the divalent cations. These results suggest that divalent ions play an important role in assembly of the ternary FGFR complex of ligand, kinase ectodomain and heparan sulfate under physiological conditions.

V-1031

Retinoblastoma Derived Growth Factor Stimulation of DNA Synthesis in Human Retinal Pigment Epithelial Cells in Culture. J.F. Tarsio. Houston Biotechnology Incorporated, The Woodlands, TX 77381; Present Address: Bio/Chem Division, Bristol-Myers Squibb Company, Syracuse, NY 13221

Purpose. Retinoblastoma derived growth factor (RBDGF) is a polypeptide secreted from the Y-79 human retinoblastoma cell line that has been shown to stimulates DNA synthesis and cell division in mouse lens epithelial cell lines. We were interested in determining if RBDGF stimulates DNA synthesis in human retinal pigment epithelial (RPE) cells. Methods. RPE cell stock cultures were established by standard techniques using human donor eyes (obtained from the Lions Eye Bank of Houston, Texas). Stock cultures were dissociated with 0.05% trypsin-0.02% EDTA and seeded at a density of 5 x 10^4 cells into 16 mm type IV collagen/laminin-coated wells in 1 ml of DMEM-F12 medium containing 5% (v/v) human serum. The cells were allowed to attach overnight, washed with medium, and then exposed to a serum-deprivation step for 42h in order to bring the cells into a resting state. Spent media was then removed and the cells incubated for another 30 h in the presence of 0.5 µCi/well of ^{3}H-thyminine and partially-purified RBDGF (obtained by concentrating Y-79 serum-free conditioned medium followed by Heparin-Affinity and S-Sepharose ion exchange chromatography). Uptake of the tracer into DNA was measured and comparisons made to the addition of DMEM-F12 medium alone or human serum. Results. RBDGF added to 50 ng/ml produced a 124% increase in DNA synthesis over that of media alone. RBDGF at 100 ng/ml produced an 104% increase in DNA synthesis. RBDGF at 5 or 25 ng/ml did not have an effect. This compared to a 9.2-fold increase in DNA synthesis produced by the adddition of human serum to 10% (v/v). However, in the latter case, the final protein concentration was 7.2 mg/ml. Conclusion. The above indicates that RBDGF is a potent mitogen for human RPE cells in culture. These results suggests that there may be some value in exploring the use of RBDGF in the treatment of retinal disorders involving the rpe cell layer.

V-1032

A Novel Serum-Free Medium for the Cultivation of Vero Cells on Microcarriers. Z. Chen, C. Xiao, H.Liu, B. Wu, X. Jia and Z. Huang. Department of Cell Engineering, Institute of Biotechnology, 23A Qilizhuang Road, Fengtai, Beijing 100071,China.

Vero cells are routinely propagated on microcarrier in serum-free containing media, to produce human viral vaccines. For cost, ease of purification and regulatory reasons,serum-free cultivation of vero cells on microcarrier is highly desirable. A novel serum-free medium for the cultivation of vero cells on microcarriers was developed, which was composed of the 1:1 mixture of DMEM/F12, BSA, EGF, gelatin and D-biotin. Both BSA and EGF were effective on cell growth, adhesion and spreading. Further addition of gelation and D- biotin led to the enhanced cell adhesion and spreading without growth promoting activit. The serum-free medium was found to bring about adhesion and spreading effects comparable with 5% FCS containing medium employed as a control, but the doubling time prolonged for about eighteen hours when vero cells were grown attached in tissue culture flasks. It also showed that the serum-free medium was suitable for the cultivation of vero cells on several different microcarriers. When vero cells and Biosilon were inoculated at 2.1 $\times 10^5$cells/ml and 30mg/ml respectively,the viable cell reached 3.4 $\times 10^6$cells/ml after 12 days cultivation.Replacing BSA with HSA to supplement the medium gave similar result, that was advantageous to the production of medical vaccines.

V-1033

Failure of CFTR Plasma Membrane Targeting of CFTR in a CF Pancreatic Duct Cell Line. C. Chemin-Thomas*, C. Gonindard*, C. Devaux†, O. Guy-Crotte°, C. Figarella° and E. Hollande*. *Laboratoire de Biologie Cellulaire, 38 rue des 36 Ponts, 31400 Toulouse, France; †CNRS URA 1455, 13326 Marseille Cedex, France; ° Groupe de Recherche sur les Glandes Exocrines, Faculté de Médecine La Timone, 13385 Marseille, France.

In some cystic fibrosis (CF) pulmonary epithelial cells, CFTR is not targeted to the plasma membrane. It remains within the endoplasmic reticulum and is degraded in a pre-Golgi non-lysosomal compartment. In ductal pancreatic cells, CFTR takes part in Cl^- and HCO_3^- ion exchanges.

In order to study the mechanisms of CFTR membrane targeting in ductal pancreatic cells, four human pancreatic cancer cell lines were used: Capan-1 homozygous for wild type CFTR, CFPAC-1 homozygous for delta F 508 CFTR, and two retroviral transduced clones of CFPAC-1 containing recombinant proviral cDNA with or without wild type CFTR (CFPAC-PLJ-CFTR-6 and CFPAC-PLJ-6, respectively).

A polyclonal antibody (ECL885) raised against a synthetic peptide corresponding to amino-acids 885-904 of an extracellular loop of CFTR was obtained. The specificity of ECL885 was verified by immunoblotting and immunofluorescence analyses.

The immunocytochemical analysis of CFTR localization in the living cells showed, by episcopic and confocal microscopy, (i) an immunoreactivity on apical plasma membranes of all polarized Capan-1 cells, (ii) a membrane labelling of some rare CFPAC-1 cells, but a cytoplasmic immunoreactivity when CFPAC-1 cells were fixed and permeabilized, (iii) an intense membrane immunofluorescence of CFPAC-PLJ-CFTR-6 cells, and (iiii) no membrane labelling of CFPAC-PLJ-6 cells.

In conclusion, we show that (i) ECL885 is a useful antibody for the study of the CFTR trafficking mechanisms, (ii) CFTR fails to target the plasma membrane of delta F 508 pancreatic ductal cells, and (iii) transfection of delta F 508 cells with wild type CFTR cDNA restores a correct membrane targeting of CFTR.

T-1001

Detecting the Transcriptional Responses to Genotoxins in Mammalian Cells with and without Exogenous Bioactivation. MD TODD, P Gee, and S B Farr. Xenometrix, Inc., Boulder, CO 80301

The purpose of the current study was to determine stress gene responses in the CAT-Tox (L) assay when human liver cells were exposed to DNA damaging agents. The CAT-Tox (L) assay consists of fourteen recombinant human liver cell lines containing stress gene- or response element-chloramphenicol acetyl transferase (CAT) constructs. A broad range of promoter and response elements was chosen for the assay including promoters responsive to DNA damage, growth arrest, tumor promotion, protein damage, and changes in intracellular cyclic AMP. Different genotoxic agents which were known to generate DNA adducts covalently (e.g. 4-nitroquinoline-N-oxide), to cross link intra- and interstrands of DNA (e.g. mitomycin C), to block replication by disrupting the stacking of bases in the DNA helix (e.g. ethidium bromide), or by inhibiting DNA synthesis (e.g. hydroxyurea) were tested to explore the range of responses in CAT-Tox (L). An important requirement for the toxic activity of many genotoxins is biotransformation. The typical endpoint measured after exogenous biotransformation is mutagenesis. In this study we measured stress gene transcriptional induction in response to toxic intermediates that were generated using rat liver S9 extract. We used a variety of biotransformation-dependent chemicals including aflatoxin B1, cyclophosphamide, and 2-aminoanthracene. Different classes of DNA damaging agents resulted in different stress gene profiles. Most genotoxins tested transcriptionally up-regulated several of the DNA damage responsive stress gene-CAT constructs such as the GADD153, GADD45, and FOS promoters, and the p53 response element. In addition to these indicators of DNA damage, other cellular responses were measured in the CAT-Tox (L) assay.

T-1002

Atypical Cytochrome P-450 Induction Profiles at the mRNA and Enzyme Level In Glomerular Mesangial Cells. A.R. PARRISH, R.C. Bowes III, M.A. Steinberg, K.L. Willett, W. Zhao, S.H. Safe, and K.S. Ramos. Faculty of Toxicology and Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, Texas.

Differential regulation of cytochrome P-450 (CYP) gene expression has been associated with cell-and species-specific differences in the metabolism and toxicity of chemical carcinogens. In the present study, the pattern of CYPIA1 gene expression and inducibility was examined in glomerular mesangial cells (GMCs), putative targets of aromatic hydrocarbon (AH) nephrotoxicity. Exposure of cultured GMCs to benzo(a)pyrene (BaP, 30 μM) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, 10 nM) for 24 hr induced *CYPIA1* mRNA levels, a response abolished by co-treatment with 10 μM cycloheximide. The pattern of inducibility was atypical in that BaP was a more effective inducer of *CYPIA1* than TCDD, a potent Ah receptor ligand, and that induction increased aryl hydrocarbon hydroxylase (AHH) activity but not ethoxyresorufin-o-deethylase (EROD) activity. Co-treatment with α-naphthoflavone (αNF, 1 μM) or ellipticine (ELLIP, 0.1 nM), known inhibitors of *CYPIA1*, partially inhibited the induction of AHH activity by BaP (30 μM). Exposure of GMCs to 30 μM BaP was associated with the occurrence of 8 DNA adducts, a response inhibited by pre-treatment with αNF (1 μM), but not ELLIP (0.1 nM). These results demonstrate that the hydroxylase activity induced by AHs in GMCs metabolizes BaP to reactive intermediates which bind covalently to DNA. The profile of induction and toxicity in GMCs is consistent with a *CYP* isoform of overlapping substrate specificity with *CYPIA1*, but differential inhibitor specificities. (Supported by grant ES 04917).

T-1003

The Cytotoxicity of 4-(Methyl-nitrosamino)-1-(3-pyridyl)-1-butanone on Pancreatic Duct Cell Cultures. M.K. REDDY, Department. of Pathology, Northwestern University Medical School, Chicago, Il 60611.

Syrian hamster pancreatic duct cells growing as monolayers on type 1 collagen gel were exposed to 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The effect of NNK on cell growth was determined by nuclear count, tritiated-thymidine incorporation into cellular DNA, and cell viability. A dose-dependent decrease in cell number and tritiated-thymidine incorporation was found in concentrations ranging from 0.1 to 50mmol/L. Cytotoxicity was evident only with a 5mmol/L or greater concentration of NNK, showing proportional decrease in cell numbers and cell viability. Morphologic features of the cells were not affected at concentrations up to 20mmol/L for up to 24 hours. However, after 24 hours, there was collagen lysis (matrix lysis), cell attachment to plastic and cell death above 20mmol/L NNK. Cytotoxicity was associated with cell cycle: an increase in S-phase cell population from 9.5% in control, up to 24% in cells exposed to 20 mmol/L NNK, and a decrease in the ratio of G_1 cells to G_2 cells was observed. Pancreatic duct cells metabolized 1 mmol/L NNK to 4-(methyl-nitrosamino-1-(3-pyridyl) butanol (NNAL). Approximately 23.4 ± 2.7 n mole of NNAL was estimated per million cells in 24 hours. Tritiated-NNK autoradiography localized NNK in cytoplasm and in the nucleus of cells after 24 hours of exposure. O^6-methyl guanine adducts were also evident in cell nuclei in a time dependent manner between 2 and 24 hour exposure and persisted for at least 48 hours after NNK was removed. The gene expression of cell cycle-related gene proliferation-associated cell nuclear antigen (PCNA) and p53 expression was decreased by exposure to NNK. The data suggest that the cytotoxicity of NNK and its interference with gene expression of p53 and PCNA may trigger initiation of pancreatic duct cell transformation *in vitro*.

T-1004

Retinol Stimulation of Clara Cell-Antigen Expression in an Epithelial Stem-Cell Line (M3E3/C3) of Syrian Hamster Lung. M. EMURA, A. Ochiai, G. Singh*, I. Hilger, S.L. Katyal* and D.L. Dungworth. Hannover Medical School, Germany, and *Department of Veterans Affairs, Pittsburgh, PA 15240

Great advances have already been achieved in the establishment of reliable in vitro lung cell models. Nevertheless, its anatomical and physiological complexity have left many important questions unanswered. The effect of retinol (VA) on the expression of Clara cell (CL-cell) antigen and carcinogen-metabolizing capacity, for example, as will be dealt with in the current paper. The cell system used was a lung epithelial stem-cell line derived from a Syrian hamster fetus on day 15 of gestation. In the previous report the morphological features developed in this cell line by VA were reminiscent of CL-cells. To substantiate this, immunostaining (I), ELISA and agarose chromatography were carried out using an antiserum for hamster CL-cell-specific 10kD protein. The major population of I-positive cells showed intracytoplasmic granular fluorescence. Such positive cells were less than 3% in the culture grown on the plastic without VA. When grown on type 1 collagen gel (CG) without VA, the positive cells were about 30%. On CG with VA (24μg/ml) they amounted to more than 70%. In this condition the CL-cell antigenicity in the secretory products detected by ELISA reached a peak on day 12 of cultivation, being over 1.5-fold higher than in the control without VA. The antigenicity in cell homogenates was virtually constant from day 6, being slightly higher with VA than without. The main components of CL-cell granules, neutral glycoproteins and specific antigenic proteins seemed to be different molecular entities. On in situ incubation with benzo(a)pyrene (BaP) on CG presumably residual VA inhibited the BaP metabolism, whereas its metabolite 7,8-dihydrodiol was clearly detected. To cope with this disadvantage of VA, the cells were released from CG to plate on the plastic and incubate with BaP. As a result, the VA-exposed cells metabolized more BaP (680pmol/μgDNA/2 days). VA may be an important factor regulating in vitro function of CL-cells.

T-1005 A new assay for toxicity of alloplastic materials

In the urinary tract: preliminary results with bladder catheters J.V.. Harney, C. E. MOTHERSILL and D. M. Murphy. Departments of Urology Beaumont Hospital, Dublin, Royal Liverpool Hospital, Liverpool and Department of Physics, Dublin Institute of Technology.

The aim of this study was to develop a technique for assessing toxicity of alloplastic materials in the urinary tract. An explant tissue culture technique was used to asses the impact of alloplastic material on growing urothelial cells *in vitro* . Specimens were prepared for tissue culture within 18 hours after excision. Using a purpose built cutting template 2 mm^2 areas of urothelium were prepared and incubated in 2 ml RPMI at 37^0C. Each catheter to be tested was incubated in RPMI for a fixed time period. Subsequently the culture medium of the cells was changed to this catheter exposed medium and test specimens were incubated in this medium for 10 days.
At the end of this time the area of urothelium covering the flask was calculated for each individual cell culture. A minimum of 8 replicate specimens were examined for each catheter at each time point. Results for catheters are expressed below as the % of control growth seen in test cultures.

Catheter	% of Control	
	1 Day	3 Day
Bard all Silicone	87.9%	105%
Argyl all Silicone	2.5%	2.3%
Eschmann all Silicone	100%	96.7%
Cliny all Silicone	109%	99.5%
Bard Silicone Elastomer	99.7%	100%
Bardex Teflon Coated	63.7%	52.7%
Bard Hydrogel Coated	110.7%	82.3%
Rusch Silkolatex	83%	90.4%
Eschmann Silicone Treated	49.5%	1.8%
Baxter Siliconized Latex	63.8%	10%

These results using normal human urothelium indicate that previous assays of toxicity using animal cells or human cancer cells underestimate the toxicity of alloplastic materials to human urothelium. Furthermore a number of catheters tested were found to be highly toxic to human urothelium.

T-1006

Selective Inhibition of Murine Prostaglandin Synthase 1 or 2 by NSAIDs Using Mammalian Cell Lines Retrovirally Infected with Murine Prostaglandin Synthase cDNAs.

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Commonly used nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and acetylsalicylic acid may cause adverse side effects in humans because they nonselectively inhibit the constitutive form of prostaglandin synthase (PHS-1) as well as the inducible form (PHS-2). Since PHS-2 modulates many inflammatory effects, inhibition of PHS-2 accounts for the therapeutic benefits of NSAIDs. Conversely, since PHS-1 regulates important cellular housekeeping functions, inhibition of PHS-1 may explain NSAIDs' undesirable effects such as gastric and renal damage. To aid in the identification of more selective NSAIDs, we have developed derivatives of mouse embryonic fibroblasts (C3H10T$^1/_2$/Cl8) and Chinese hamster ovary (AS52) cells that stably express high levels of either murine PHS-1 or PHS-2 using packaged retroviral vectors. Acetylsalicylic acid, indomethacin, ibuprofen, naproxen and NS-398 (kindly supplied by Taisho Pharmaceutical Co., Tokyo, Japan) were tested in both cell types. Of the five, indomethacin was the most potent inhibitor of both isozymes and inhibited PHS-2 only slightly better than PHS-1. While acetylsalicylic acid preferentially inhibited PHS-1, ibuprofen, naproxen, and NS-398 preferentially inhibited PHS-2. Of the latter three, NS-398 was the most potent (IC_{50}s of 2×10^{-7} and 3×10^{-8} M for PHS-2-expressing 10T$^1/_2$ and AS52 cells, respectively) and exhibited the greatest differential inhibition of PHS-2 relative to PHS-1, i.e. NS-398 had no effect on PHS-1 over the range of doses tested (10^{-4} to 10^{-11} M). These data show how cell lines such as these provide good tools for the identification of selective and therefore, safer NSAIDs.

T-1007

Stripping and reconstitution of HLA class I associated peptide for generation of antigen-specific CD8+ T-lymphocytes.

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Elicitation of HLA class I mediated CD8$^+$ CTL response to immunodominant peptidic antigens often requires stimulation with potent antigen presenting cells (APC). We have demonstrated the ability to generate HLA class I restricted CD8$^+$ CTL from peripheral blood of non-immunized HLA-A*0201 individuals without the need to isolate APC. Whole blood or peripheral blood mononuclear cells (PBMC) was subjected to mild acid treatment (pH ~3.3) in an iso-osmolar buffered environment. Combinations of acids and buffers were evaluated for their ability to strip HLA class I associated peptide without loss of cell recovery and viability. Acid stripped cells remained viable and were phenotypically characterized to be HLA class I deficient using W6/32, an antibody recognizing HLA class I heavy chain associated with peptide and ß2-microglobulin. Stripped cells were reconstituted with a nine amino acid HLA-A*0201 restricted peptide from the pol region of HIV in the presence of ß2-microglobulin and cultured with PBMC to generate peptide-specific T-lymphocytes. Lymphocytes were expanded according to a restimulation schedule employing peptide-pulsed autologous PBMC and low dose IL-2 to numbers in excess of 50×10^6 cells after 4 weeks of culture. Obtained CTL exhibited antigen-specific lysis of peptide pulsed target cells in a dose-dependent fashion in *in vitro* ^{51}Cr release cytotoxicity assay. This antigen-specific killing was shown to be HLA class I restricted. We have thus demonstrated the ability to generate and expand antigen-specific CD8$^+$ CTL employing acid treatment and peptide reconstitution protocols without the need for elaborate and intensive procedures to isolate and purify antigen-presenting cells, such as dendritic cells. This procedure is simple, efficient and can be performed in a closed system for clinical applications in adoptive immunotherapy.

T-1008

Development of a Fluorescence Based Amino Acid Analysis System Suitable for Analysis of Tissue Culture Media and Cell Culture Broth.

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With several thousand dollars at stake every time the modern biotechnology fermenter is loaded, thorough analysis of the media constituents is prudent. Analysis of the amino acid (AA) constituents has long been problematic due to the presence of many other compounds which may interfere with derivatization or detection of the AA derivatives. A recently described method of precolumn derivatization amino acid analysis (AAA) using 6-aminoquinoly-N-hydroxysuccinimidyl carbamate (AQC) has been shown to provide excellent accuracy and precision when used for the analysis of protein hydrolysates. Because hydrolysate methods were developed for samples lacking critical AAs found in tissue culture media, e.g., Asn, Gln, Hypro, etc., new methods were requested. The following presentation will describe adjustments made to various parameters including; pH and ionic strength of the mobile phase, column temperature, and gradient slope, to achieve the desired separation. Examples of media and cell culture broth analysis will be used to demonstrate the system's ability to analyze these complex samples.

T-1009

Serum-Free Production of Rotaviruses
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Rotaviruses are the most common cause of acute infantile gastroenteritis. In the U.S. alone, it has been estimated that 100,000 hospitalizations and 150 deaths of children under age 3 result from rotavirus infections annually. Oral administration of a bovine-human rotavirus reassortant vaccine has been shown to be well tolerated and to protect against severe gastroenteritis caused by wild-type organisms (Christy, C. et al. 1993). Production of rotaviruses for use in an oral vaccine is complicated by the requirement of a proteolytic cleavage of the VP4 surface protein to achieve infection of Vero cells in vitro. A serum-free process is preferable since fetal bovine serum quenches the proteolytic activity of trypsin, making extensive washing of cells grown in serum-containing medium necessary prior to infection. We have developed a serum-free, low protein medium capable of supporting 1) Vero cell growth for up to 20 passages, 2) Vero cell growth on microcarriers,and 3) rotavirus production. Maximum Vero cell density attained in this medium is about 50% of that seen in serum-containing medium and the doubling time is 36 h, compared to 24 h for serum containing medium. Process improvements to date have eliminated the lengthy wash steps associated with the serum-containing process and have maintained the same virus titers. Rotavirus production in depleted serum-free medium is lower than in completely fresh medium, indicating a nutrient limitation or an end product inhibition of virus production. Work is ongoing to further define this limitation.

T-1010

Identification and Characterization of the Extracellular Matrix Molecule Restrictin. J.J. HEMPERLY, R.L. Ackley, and R.A. Reid. Becton Dickinson Research Center, P.O. Box 12016, Research Triangle Park, NC 27709.

Restrictin, also known as janusin or J-160/180 in rodents, is an extracellular matrix molecule originally identified in the nervous system. Depending on cell type and presentation context, restrictin can have both supportive and inhibitory effects on neurite outgrowth. The human homolog of restrictin has been identified and its complete cDNA sequence determined. Rabbit antibodies were prepared against a fragment of human restrictin expressed as a GST fusion protein in bacteria. These antibodies, in addition to detecting the immunogen and high molecular weight polypeptides in human brain, detect a 170 kD polypeptide in MatriGel, an ECM product of rat EHS sarcoma cells widely used as a tissue culture growth substrate. Moreover, the antibody immunoreactivity can be blocked with restrictin fragments expressed in bacteria, but not by GST protein alone. This suggests that MatriGel may be a source of restrictin for use as an alternative tissue culture substrate or that the growth-promoting activity of MatriGel may be modulated by changing its restrictin composition.

T-1011

Hematotoxic Effects of Chemotherapeutic Drugs Assessed Using Three-Dimensional Bone Marrow Cultures. J. SAN ROMÁN, V. Kamali, B. Sibanda, J.M. Gee, and B.A. Naughton, Advanced Tissue Sciences Inc., La Jolla, CA 92037.

Hematotoxicity is associated with exposure to chemotherapeutic drugs and numerous other agents. We tested the influence of drugs on hematopoiesis in three dimensional co-cultures of rat bone marrow (BM) stromal and hematopoietic cells. These co-cultures maintain multilineage hematopoietic expression (*i.e.*, myeloid, monocytic, erythroid, monocytic) for extended periods and display continuous proliferation of colony forming unit-culture (CFU-C) hematopoietic progenitor cells. The stereotypic microenvironment promotes the development of normal cell-cell interactions and enhances the exposure of developing hemato-poietic cells to growth/regulatory factors elaborated by the stromal cells as well as nutrients. After 40 days *in vitro*, BM co-cultures were incubated with agents that exhibit differing mechanisms of hematotoxicity including: cyclophosphamide (CP), 4 hydroperoxycyclophosphamide (4-HC), vincristine , chloramphenicol, daunorubicin, 1,3 *bis*(2-chloroethyl)-1-nitrosourea (BCNU), or toluene. Adherent zone cells were harvested, phenotyped by flow cytometry and assayed for CFU-C content. BCNU, daunorubicin, chloramphenicol, and toluene exposure resulted in a dose-related diminution in CFU-C numbers in the adherent zones of BM co-cultures. Cytofluoro-graphic analyses revealed that certain drugs displayed lineage specificity: *e.g.* vincristine , daunorubicin and BCNU exhibited greater toxicity to myeloid cells than B lymphocytes at the dose levels we employed. Additionally, CP exposure caused dose-related decreases in CFU-C numbers; 4-HC, the active form of CP, exacerbated this effect, decreasing CFU-C progenitors by >70% at doses $\geq$176 ng/ml as compared to non-treated controls. The use of cultured BM to assess hematotoxicity *in vitro* may be a useful tool to screen developmental drugs prior to commitment to full-scale animal testing.

T-1012

IL-4 Production using macroporous microcarriers
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Cytopore™ (distributed as Asahi microcarrier in Japan) is a new macroporous microcarrier made of cellulose substituted DEAE. It is designed to produce a high density cell culture over a long period. We found a method for counting total cell numbers attached to Cytopore™ with enzymes to dissolve the cellulose matrix and extracellular matrix, and confirmed with a confocal microscope that cells are viable at the center part of Cytopore™. Comparing the productivity of recombinant cells, recombinant CHO-producing IL-4 was cultivated both in suspension and microcarrier culture. High cell density was easily maintained when CHO cells were immobilized in Cytopore™. The IL-4 productivity of microcarrier culture was higher than suspension culture when in serum-free conditions.

T-1013

Subculture method for large scale cell culture using macroporous microcarrier. *K.Kamiya, K.Yanagida, J. Shirokaze. Cellulose Fibers Department, Asahi Chemical Industry Co., Ltd., 4-3400-1, Asahi-machi, Nobeoka City, Miyazaki 882, Japan. *Fuji Pharmaceutical Plant, Asahi Chemical Ind. Co., Ltd., 2-1, Samejima, Fuji, Shizuoka, 417, Japan

Cytopore™ (distributed as Asahi microcarrier in Japan) is a new macroporous microcarrier made of cellulose. It was developed for high density cell culture and is expected to be used for large scale production of bioactive substances. But a subculture method, which is essential for large scale cell culture, has not been established yet. So we have developed a subculture method using macroporous microcarriers. We have confirmed that this method is useful not only with r-CHO cells but with BHK-21, HeLa, Vero and hybridomas. This method is characterized by not needing trypsinization, and has many advantages in producing bioactive substances.

T-1014

A Novel Method for the Analysis of Amino Acids in Cell Culture Media. J.M. KUBIAK[1], C. Van Wandelen[2] and S.F. Gorfien[1]. [1]Cell Culture R&D, Life Technologies, Inc., Grand Island, NY 14072. [2]WATERS Corporation, Milford, MA 01757.

During the development and optimization of cell culture medium, analysis of nutrient composition is a valuable tool. Amino acids are key components of cell culture media. Therefore, the concentrations of these components in the medium are useful indicators of the physiological status of a culture. Existing protocols for routine amino acid analysis suffer from multiple limitations. Some of these limitations include poor intermediate or derivative stability, chromatographic matrix effects or lack of assay reliability in evaluating a complete group of primary or secondary amines.

The method described uses 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) as the precolumn derivatizing agent. The derivatization procedure was previously described by Cohen et. al. (Anal. Biochem. 211:279-287; 1992). We have modified this procedure for analysis of amino acids in cell culture media.

Amino acid concentrations were determined using this protocol for several traditional serum supplemented and serum free formulations. All amino acids of interest could be quantified in one assay. Quantitative results suggest comparability to current o-phthalaldehyde (OPA) and phenylisothiocyanate (Pico-Tag®) assays conducted by this laboratory. Major advantages of the AQC procedure are greatly reduced sample preparation time, increased sample stability and simplicity as compared to other routinely used assay procedures. An additional benefit of this method is the ability to quantitate free ammonia, a common factor which limits growth and cellular productivity in cultured cells. The availability of simple reproducible methods for quantitation of key nutrients will facilitate investigation of cellular physiology. The AQC method should be considered an important tool for the academic researcher as well as the industrial process development scientist.

T-1015

Sensitivity of Isoenzyme Analysis for the Detection of Cell Line Cross-Contamination. R.W. NIMS, A.P. Shoemaker, M.A. Bauernschub, and J.W. Harbell. Microbiological Associates, Inc., Rockville, MD 20850

The analysis of the gel electrophoresis banding patterns and relative migration distances for the individual isoenzymes of lactate dehydrogenase (LD), purine nucleoside phosphorylase (NP), glucose-6-phosphate dehydrogenase (G6PD) and malate dehydrogenase (MD) is used routinely in the biopharmaceutical industry for confirmation of cell line species of origin. In the present study, the sensitivity of the technique (AuthentiKit™, Innovative Chemistry) for determining cell line cross-contamination was examined. Extracts were prepared from CHO-K1 (hamster), MRC-5 (human) and L929 (mouse) cells and from various proportional mixtures (1:20, 1:9 and 1:2) of the combinations of cells. The isoenzymes were analyzed according to standard procedures for the technique. Contamination of MRC-5 cells with CHO-K1 or L929 cells was clearly detectable with each enzyme analyzed. Similarly, the contamination of L929 or CHO-K1 cells with MRC-5 cells was readily apparent with each enzyme. On the other hand, contamination of CHO-K1 cells with L929 cel was only detected with LD analysis, and contamination of L929 cells with CHO-K1 cells was not detected with any of the four enzymes examined. For the latter case, the addition of other enzymes (eg., peptidase B) to the battery of enzymes would be required.

T-1016

Cells on Rotating Fibers. R.CLYDE, POB 740644, New Orleans, La.70174

A small, inexpensive reactor has been developed for optimization studies of plant and animal cells. A magnet is near (but not touching) the bottom. There is no rotary seal on top that can leak and the reactor is full of fibers.
In another design, corrugated fibers are rotated in a horizontal, half full reactor Oxygen can be supplied at an end and also in the middle. Liquid is carried up into the vapor space and drops down through holes in the valleys. Mass transfer to drops is much faster than to a flat surface. When oxygen is applied in a vertical reactor, it can become depleted as it passes through the fibers.

T-1017

Photoregulation, Purification and Application of B-phycoerythrin from *Porphiridium cruentum*. K.MINKOVA**, A.Tchernov*, E.Dimitrova*, A.Mihova*, **Institute of Plant Physiology, Bulgarian Academy of Science, *National Centre of Infectious Diseases, Sofia 1113, Bulgaria

B-phycoerythrin (B-PE) is a widely used fluorescein dye for mono- and multi- colour labeling. We studied a new method for biomass productivity of B-PE from red alga *Porphiridium cruentum* by illumination of the algae by red light. B-PE was exracted from the algal cells with 0,01M K-phosphate buffer (pH 7,0) and purified with rivanol. The crude extract was saturated with $(NH_4)_2SO_4$ several times. Collected supernatants were mixed, acidified with 0,1 M acetic acid to pH<5 and applied to a column of Sephadex G-25. The absorbtion spectra of the fractions were determined. B-PE fraction was subjected to gel-filtration on Sepharose CL-6B. Monoclonal antibodies (Mabs) against human leucocytes membrane receptors were conjugated with B-PE by maleimid-suucinimid method. The conjugates were purified by gel-filtration and their activity and specificity was studied by flow cytometry.

T-1018

Production of an In Vitro Reconstituted Skin using Human Neonatal Foreskin Keratinocytes (HFK) in Combination with the Dermal Substrate AlloDerm®. E.S. GRIFFEY[1] and S.A. Livesey[2]. [1]Baylor College of Medicine, Center for Biotechnology, The Woodlands, TX 77381 and [2]LifeCell Corp., The Woodlands, TX 77381.

Studies have been performed to evaluate different techniques for culturing human neonatal foreskin keratinocytes (HFK) onto an acellular dermal matrix (AlloDerm). AlloDerm is human skin processed to remove cells of the epidermis and dermis while leaving the structural matrix of the dermis and basement membrane complex (BMC) intact. Immunohistochemical studies were performed to confirm retention of BMC proteins including collagen types IV and VII, laminin and heparin sulphate proteoglycan. AlloDerm has also been shown clinically to be immunologically inert and hence appears to be an optimal matrix for the in vitro production of a reconstituted skin suitable for grafting. Studies were performed to optimize the producion of an in vitro reconstituted skin. In this work co-cultures were established using keratinocytes isolated in different mediums and cultured in the presence or absence of various agents (e.g. retinoic acid, increased calcium levels, serum supplementation) known to regulate the growth of HFK. The resulting co-cultures were evaluated by immunohistochemical localization of different cytokeratins including K5, K14 and K10. We have found that keratinocytes isolated and propagated in the absence of serum produce a truncated pattern of differentiation when placed in co-culture with AlloDerm. In contrast, when HFK were isolated and propagated in the presence of serum a more normal pattern of differentiation resulted. These results were confirmed by cytokeratin staining. These studies have resulted in the production of an in vitro reconstituted skin which exhibits cytokeratin expression similar to skin in vivo.

This research was supported by the National Science Foundation under award number III-9361046.

T-1019

In Vitro Cytotoxicity Testing of Potentially Active Anti-HIV Drugs with Cultured Cells. F.A. BARILE, D. Hopkinson and P. Scheiner. Department of Natural Sciences, City University of New York at York College, Jamaica, NY 11451.

This study was designed to compare two continuous cell lines in culture for their ability to determine cytotoxicity of potentially active anti-HIV drugs. Human fetal lung fibroblasts (HFL1) and T4 lymphocytes (CEM, NCI Labs) were incubated in the absence or presence of increasing concentrations of 12 antiviral compounds synthesized in our labs. Incubations were continued for 6 days and mitochondrial activity (XTT and MTT assays) was used as a marker for toxicity. Inhibitory concentrations 50% (IC50s) were extrapolated from concentration-effect curves after linear regression analysis. Comparison of the two sets of cytotoxicity data suggests that the experimental IC50 values from HFL1 cells correlate well with lymphocyte studies performed at the NCI (r value = 0.93). In addition, the cell lines revealed similar cytotoxic responses, supporting our basal cytotoxicity hypothesis (Barile *et al.* CBT 10:155, 1994). It is anticipated that these procedures may supplement or replace currently used animal protocols to screen therapeutic drugs for human risk assessment. (Supported in part by NIGMS GM08153).

T-1020

Agar Diffusion Cytolysis and Aqueous pH: A Classification Algorithm of Two *In Vitro* Tests for *In Vivo* Ocular Hazard Categorization. D.A. LASKA, W.P. Hoffman, and J.T. Reboulet. Toxicology Research Laboratories, Lilly Research Laboratories, Greenfield, IN 46140

The agar diffusion cytolysis method has been used in this laboratory as an ocular irritation screen for several years. The correlation between *in vitro* lytic response, at a dose of 25 mg or 25 µl, and the maximum corneal opacity score *in vivo*, 24-72 hours following a single test article treatment, has been excellent (98.5%) for test articles between pH 4 and pH 9 (when pH was measured in water). However, a significant number of test articles encountered routinely have an aqueous pH of ≤ 4.0 and ≥ 9.0. The agar diffusion matrix, composed primarily of buffered culture medium, was believed to mask the corneal damage potential of acidic or caustic test materials. Consequently, the purpose of these experiments was to combine lytic zone size and aqueous pH in an algorithm to arrive at an ocular irritation classification. The data consisted of 93 unique test articles with various *in vivo* ocular irritation scores, and which ranged in pH from 2.6 to 11. The lytic zone size, following 24 hour incubation of the test article with the test cultures, ranged from 0.0 mm to 100 mm. Assay sensitivity and specificity, based on a two category scheme (severe or non-severe) were 92.6% and 92.4%, respectively. Furthermore, using this algorithm, we were able to correctly classify 74.2% of the 93 test articles as either non-irritating, irritating, or severely irritating. The results from the combination algorithm represent a major improvement in classifying the ocular hazard potential of all solid or liquid test articles, regardless of their solubility properties or pH in water, versus the results from either *in vitro* test evaluated alone.

T-1021

Rabbit Corneal Epithelial Cell Lines as an *In Vitro* Alternative Model for Evaluating The Efficacy and Cellular Toxicity of Drugs: I. Immortalization and Preliminary Characterizations. C. YAO, D. Wampler, D. Grimm, K. Hall, D. Shade, D. Crouch, J. Veltman and R. Hackett Departments of Research Toxicology and Molecular Pharmacology, Alcon Laboratories, Inc., Fort Worth, TX 76134

Rabbit corneal epithelial cell lines were established by the transfection of serum-free primary cultured corneal epithelial cells with origin defective Simian virus (*Ori*-SV40, clone 6-1) DNA. Indirect immunofluorescence studies show that these cells express cytokeratin intermediate filaments, AE3 and AE5, but not vimentin. These cell lines express SV40 large T antigen in their nuclei. SEM analysis shows that these cells have microplicae on cell surface. Mean estimated cell diameter ranges from 25 to 50 um. These cells exhibit cobble stone epithelial cell morphology in serum-free media. TEM analysis showed that these cells grew in monolayer at subconfluent stage and formed multilayers at overconfluent stage. Average plating efficiency is about 30% and the average population doubling time is 18.3 $\pm$ 2.0 hrs. Pharmacological characterization demonstrates the presence β-adrenergic, prostaglandin EP, histamine, dopamine, and adenosine receptors. The activities of lactate dehydrogenase, glucose-6-phosphate dehydrogenase and aldolase were 16.8 $\pm$ 0.5 to 29.9 $\pm$ 4.9 umol/mg/min, 471.2 $\pm$ 60.0 to 769.9 $\pm$ 119.8 nmol/mg/min, 637.4 $\pm$ 48.2 to 821.3 $\pm$ 28.8 nmol/mg/min, respectively. Glutathione analysis showed a range of basal level from 42.0 to 131.7 nmole/mg. Results indicate that these cell lines may be useful models for cellular toxicity and efficacy screening of ocular drug candidates.

T-1022

Immortalized Lens Epithelial Cells as an *In Vitro* Model for The Efficacy and Cellular Toxicity Evaluations of Ocular Drugs: I. Immortalization of Cells and Preliminary Characterizations. D. Wampler, D. Grimm, Guo-Tung Xu*, D. Shade+ R. Hackett and C. YAO Departments of Research Toxicology, Degenerative Disease* and Molecular Pharmacology+, Alcon Laboratories, Inc., Fort Worth, TX 76134

Rabbit lens epithelial cell lines were established by transfection of primary lens epithelial cells with origin defective Simian virus DNA (*Ori*-SV40). All established lines possess SV40 large T antigen in their nuclei and express cytokeratin AE5 and vimentin intermediate filaments in their cytoskeleton. Staining with anti-α crystallin monoclonal antibody shows that these cells do express α-crystallin, a specific marker for lens epithelial cells. Results from gel electrophoresis with crystallin standards confirmed the presence of both α- and β-crystallins in lysates from these cells and from fresh lens epithelial cells. In serum-free DF-12 medium , cells developed into multilayers at overconfluent stage. Average plating efficiency for lines T1 and T3 are 42.8% and 35%, respectively. The average population doubling time for T1 and T3 in DF-12 medium are 16.0 $\pm$ 2.2 and 18.51 $\pm$ 3.54 hrs, respectively. Pharmacological characterization shows that T1 line express β-adrenergic, prostaglandin EP, histamine, and adenosine receptors based on adenylate cyclase activities stimulated with different known pharmacological agonists. Results indicate these cells do retain their key differentiation characteristics and may be useful for *in vitro* efficacy and cellular toxicity evaluations of ocular drug.

T-1023

A Human (HepG2) Cell Line Model For Cadmium Toxicity Studies. P.F. DEHN, C.M. White, D.E. Conners, G. Shipkey, and T.A. Cumbo. Biology, Canisius College, Buffalo, N.Y. 14208

Both biochemical indicators and in vitro models for toxicity testing offer potentially sensitive tools for inclusion into toxicity assessment programs. In vitro models must mimic in vivo responses to provide meaningful data. This study was designed to investigate the utility of the HepG2 cell line in mimicking known in vivo responses of mammalian systems when confronted with cadmium (Cd^{+2}). Uptake of Cd^{+2} and several cytotoxic and stress effects were measured in both naive and Cd^{+2} pre-adapted cells: viability (neutral red uptake, MTT dye conversion, Live/Dead) membrane damage (lactate dehydrogenase leakage), metabolic activity (ATP production), and detoxification capabilities (glutathione content, EROD activity, and metallothionein [MT] induction). Uptake and all biochemical responses except EROD and MT induction showed dose-response relationships, after 24 hrs exposure to Cd^{2} (ranges 0 to 3 ppm). Cadmium effects were reduced in pre-adapted cells, indicating adaptive tolerance had occurred. Twenty-four hr LC50 values were 0.473 ppm (4.18 uM) for naive cells and 0.84 ppm (7.47 uM) for pre-adapted cells based on the Live/Dead assay. The critical concentration for developing nephropathy in humans is 200 ug/g. The HepG2 cells show a cytotoxic response well below these levels. MT induction (9-fold increase) occurred in response to $ZnCl_2$, but not in response to Cd^{+2}. These data indicate that this carcinoma cell line is a useful in vitro model for cadmium toxicity studies. (Supported by NIH (TOX (AHR-A)1 R15 ES05444-01)to PFD, Hearst Foundation & HHMI grants to Canisius for undergraduate researchers CMW, DEC, GS, & TAC.)

T-1024

Comparison of pH 6.70 SHE and Balb/c-3T3 Transformation Assays to Ames and Rodent Bioassay Results. R.M. BRAUNINGER[1], G.A. Kerckaert[2] and R.A. LeBoeuf[2]. Hazleton Washington[1] 9200 Leesburg Pike, Vienna, Va. 22182. The Procter & Gamble Company[2] PO Box 398707 Cincinnati, OH.45239

A direct comparison was made between our results obtained using the pH 6.70 SHE transformation assay and literature survey results of the Balb/c-3T3 transformation assay in relation to the NTP Rodent Bioassay and the Ames salmonella reversion assay. Although each of the transformation assays have been extensively evaluated, a subset of 33 chemicals (17 carcinogens and 16 noncarcinogens) was tested in both systems. When this subset was compared to the rodent bioassay results, the pH 6.70 SHE assay correctly identified 13/17 carcinogens (76% sensitivity) and 13/16 noncarcinogens (81% specificity) for an overall concordance of 79%. The Balb/c-3T3 assay correctly identified 9/17 carcinogens (53% sensitivity) and 10/16 noncarcinogens (63% specificity) for an overall concordance of 58%. The Ames assay correctly identified 6/17 carcinogens (35% sensitivity) and 14/16 noncarcinogens (88% specificity) for an overall concordance of 61%. Finally this direct comparison between both transformation systems also included 11 "nongenotoxic" (Ames negative) carcinogens: The pH 6.70 SHE assay was able to detect 7/11 (64% sensitivity) whereas the Balb/c-3T3 assay detected 4/11 (36% sensitivity) of these nongenotoxic carcinogens. However when the data set was broadened to include nonoverlapping evaluations, both transformation systems showed an enhanced sensitivity (76% for SHE and 64% for Balb) for detecting Ames negative carcinogens. Overall this review indicates that the pH 6.70 SHE assay shows both greater specificity (81% vs 63%) and sensitivity (76% vs 53%) compared to the Balb/c-3T3 assay for those chemicals tested in common. In addition these data suggest complementation between the pH 6.70 SHE assay and the Ames assay for detecting rodent carcinogens.

T-1025

Communication by Keyword: Enhanced storage, retrieval and dissemination of information about in vitro technologies. D.J. HUGGINS. Toxicology Consulting Services, Princeton, NJ 08542

The development of more facile modes of communication, such as the Internet, and the increased use of computerized databanks for storage of information about in vitro techniques suggest that methods designed to enhance the storage, retrieval, and dissemination of this information are worthy of consideration. This poster describes one such method, a keywording strategy, which, when applied appropriately, should enhance the communication of many of the concepts emerging in the field of in vitro technologies. A small databank of articles about alternatives to skin irritation testing was constructed with the aid of commercially-available software (PRO-CITE^{tm}). Articles from 17 scientific journals were collected, keyworded according to the proposed strategy and entered into the databank. The keywording strategy included words for toxic insult(s) studied, method(s) used, endpoint(s) measured, and chemical(s) studied. A vocabulary of approximately 200 keywords emerged which reflect the nomenclature currently in use in this field. Application of such a keywording strategy should result in enhanced communication of data about in vitro technologies.

T-1026

Effect of External Ligands on Interaction of $CdCl_2$ with Cell Lines of Different Tissue Origins. J.T. JONES, D.E. Carter and H.E. Laird II,. Department of Pharmacology and Toxicology, University of Arizona, Tucson AZ 85721

Cadmium is an environmental pollutant that can be ingested from water or plant sources, or inhaled from cigarette or industrial smoke. Low level exposure has been shown to have a variety of health effects, including liver and renal toxicity and renal and prostatic carcinogenesis. This study uses continuous cell lines derived from putative target organs of cadmium toxicity. We have determined the toxicity of $CdCl_2$ for the ARL18 cell line, derived from rat liver, and the LLCPK1 line, derived from pig kidney proximal tubule, using the XTT assay. Toxicity is affected by the presence of serum, by the cell culture medium in which the assay is done, and by the presence of the external ligand bovine serum albumin. Glutathione has little effect on toxicity. In DMEM/F12 culture medium, $CdCl_2$ is more toxic to $LLCPK_1$ cells than to ARL18 cells. Cells were grown on permeable supports in order to study uptake of $^{109}CdCl_2$ into cells, and transfer of the isotope across the cell monolayer. ARL18 cells take up less of the isotope than do $LLCPK_1$ cells. However, more of the isotope is transferred across ARL18 monolayers than across $LLCPK_1$ monolayers. The pattern of uptake differs: $LLCPK_1$ cells take up more $CdCl_2$ from the basolateral side, while ARL18 cells take up more from the apical aspect. The influence of BSA on the pattern of uptake has been studied, and is complex. These studies suggest that differences in the accumulation and toxicity of $CdCl_2$ in different organs of the body may in part be due to differences in the way it interacts with the cells within the organs. (Supported in part by NIEHS grant 04940)

T-1027

In vitro Endoreduplication of Chromosomes by Organomercurials in CHO cells. AARON WILSON, Lynn Carleton, Elora Alauddin and T.S. Kochhar. Kentucky State University, Frankfort, KY 40601.

Analyses of chromosome changes is a rapid way of assessing genetic damage caused by hazardous agents present in the environment. In the present study, we investigated the effect of two organomercurials namely, phenylmercuric chloride and ethylmercuric chloride on the induction of chromosome aberrations (CA) and sister-chromatid exchange (SCE) in cultured Chinese hamster ovary (CHO) cells. The cultures were treated with 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5}M of the test compounds for 24 hr at 37°C. It was noticed that both phenyl and ethylmercuric chloride elevated the frequency of CA. Endoreduplication of chromosomes was a common abnormality and was most noticeable in 10^{-5}M phenylmercuric chloride. The compounds also enhanced SCE frequencies but less so compared to CA. The chromosome changes brought about by organomercurials may be related to ability of these agents to bind with thiol groups in cell's spindle apparatus.

(Supported by NIGMS-MBRS Grant# S14 GMO5231)

T-1028

Phosgene-induced Calcium Changes in Pulmonary Artery Endothelial Cells. R. J. Werrlein, S. D. Kirby and J. MADREN-WHALLEY. Pharmacology Division, U. S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5425.

Sheep pulmonary artery endothelial cells (PAECs), grown as attached cultures in glass bottomed microwells, were preloaded with Indo-1AM and exposed to phosgene in an environmentally controlled chamber. Using confocal laser microscopy and XZ-image scanning techniques, 2-dimensional images (cross sections) of intracellular calcium were obtained. Cells were excited with a 355 laser line and emissions from calcium-free Indo-1 (485 nm) and calcium-bound Indo-1 (405 nm) were recorded in real time. Ratio analyses of the fluorescent images obtained during 20-minute exposures showed that calcium responses to sub-lethal doses of phosgene occurred within 30-45 seconds of injecting the gas into the chamber. Doses of 98 to 147 ppm•min induced sudden 2- to >5-fold increases of intracellular free calcium and corresponding activity changes of 70 to 386 nM. Some cells produced calcium spikes with a rise time of less than 15 seconds and a recovery of 2 to 4 minutes. In others, the rise time lasted 2 to 3 minutes and peak activity lasted up to 6 minutes. As many as 3 complete calcium spikes were recorded from a single cell during one 20-minute exposure. Control populations, exposed to an equivalent volume of the carrier gas (room air), showed no comparable changes in calcium activity. We suggest that early and substantial calcium responses to phosgene may precede and contribute to phosgene's disruption of F-actin in the same cells (Werrlein et al. Cell Biol. Toxicol. 1994;10: 45-58).

T-1029

2,2 Dichlorodiethyl Sulfide (Sulfur Mustard, SM) Causes Cleavage of Human Lymphocyte DNA and Poly (ADP-Ribose) Polymerase Inhibitors (PADPRPI) Alter the DNA Patterns. H. L. MEIER and C. B. Millard, USAMRICD, APG, MD 21010.

Previously, we demonstrated that SM causes a concentration-dependent decrease in human lymphocyte cell viability, NAD, and ATP levels. PADPRPI can reduce these effects of SM by 100 fold. The relationship between the loss of viability and DNA damage is being investigated by flow cytometry and gel electrophoresis. Both techniques show a SM concentration-dependent fragmentation of the DNA. At 10^{-3}M SM, where approximately 50% of the cells are still viable by propidium iodide exclusion, both flow cytometry and gel electrophoresis demonstrate a uniform shift of the DNA pattern. In 1.5-3.0% agarose gels, the DNA pattern of the SM exposed cells is characteristic of a necrotic lesion; the DNA is heterogeneously degraded and moves further into the gel compared to the control. If PADPRPI are added with SM, a ladder pattern similar to that seen in apoptic death results, even though about 90% of the cells are viable. In similarly treated cells assayed by flow cytometry, the DNA shows a shift of the DNA peak to the right, proportional to the SM concentration. This shift is decreased by the presence of PADPRPI. PADPRPI can prevent biochemical and membrane changes induced by SM. The inhibitors do not prevent SM-induced DNA damage, but they can alter the pattern of damage.

T-1030

DNA Damage Caused by Influenza Virus-Single Cell Electrophoresis Assay. M.V.RAMANA; Y.L.Ahuja and Gita Sharma. Dept. of Microbiology, Osmania University, Hyderabad - 500007.

A high incidence of chromosomal aberrations have been reported in the germ cells of mice infected with different strains of influenza virus. In vitro lymphocyte cultures treated with influenza virus (A_2 HK H_3 N_2) also showed chromosomal abnormalities and high frequency of sister chromated exchanges. DNA damage has been evidenced by single cell electrophoresis. Details of these findings will be discussed.

T-1031

The Role of Glutathione in Protecting against Menadione-induced Cytotoxicity in Platelets Isolated from Rats. Y.S. Cho, K.S. Park, J.Y. Lee, M.J. Kim and J.H. Chung. College of Pharmacy, Seoul National Univ., Seoul 151-742 Korea

Our previous studies demonstrate that menadione is cytotoxic to platelets by oxidative stress. In order to elucidate the mechanism of this toxicity, glutathione (GSH) depletion by menadione was studied using platelet-rich plasma isolated from rats. Treatment with menadione resulted in rapid dose-dependent depletion of GSH, suggesting that GSH may have a vital role in protecting against menadione-induced cytotoxicity. Consistent with this finding, cysteine and dithiothreitol prevent menadione cytotoxicity in a dose-dependent manner, as determined by lactate dehydrogenase leakage and change in turbidity. When platelets were treated with 1-chloro-2,4-dinitrobenzene (CDNB) and diethylmaleate (DEM), both of which deplete intracellular GSH, menadione toxicity was potentiated in the CDNB-treated platelets, but not in the DEM-treated platelets. Serotonin release from the dense granule in platelets could be another cytotoxicity indicator, implying damage to intracellular organelle membranes. Menadione treatment increased serotonin release significantly. The menadione-induced serotonin release was inhibited by dithiothreitol and accelerated by CDNB treatment. These data suggest that the GSH levels in platelets plays an important role in menadione-induced cytotoxicity.

T-1032

Comparative Heavy Metal Cytotoxicity to Established Fish Cell Lines. H. SEGNER. Department of Chemical Ecotoxicology, Centre for Environmental Research, D-04318 Leipzig, Germany

Cytotoxicity tests utilizing established fish cell lines have been suggested as a screening tool to assess the potential hazard of environmental chemicals to aquatic biota. To date, however, insufficient knowledge exists on the relative sensitivity of fish cell lines towards different classes of chemicals or different modes of toxic action. The present study compares the acute cytotoxicities of cationic metals to five cell lines originating from different fish species: R1 cells (rainbow trout; own data), CHSE cells (chum salmon embryo; own data), RTG-2 cells (rainbow trout; own data), BF-2 cells (bluegill sunfish; Babich et al. 1986), and FHM cells (fathead minnow; Brandao et al. 1992). The neutral red uptake inhibition assay was used as cytotoxic endpoint. The five cell lines responded similarly with respect to the absolute concentrations of heavy metals causing a 50 % reduction in neutral red uptake. Moreover, the rank order of cytotoxicity was identical for all cell lines: silver > mercury > cadmium > zinc > copper > nickel > lead. Thus, despite different species origin, different culture media and incubation temperatures, the fish cells showed close in vitro - in vitro correlations, with r-values not lower than $r = 0.98$. On the contrary, the correlation to acute fish lethality data was clearly worse, with r-values ranging from $r = 0.64$ to $r = 0.92$. The close agreement among the in vitro data indicates that the cellular toxicity of cationic metals depends on a common structural or physicochemical determinant. A candidate factor is the chemical softness parameter σ - a measure of the polarizability of the electron cloud of metal cations - which changes in the same rank order as heavy metal cytotoxicity.

T-1033

Immortalization and Depolarizing Conditions Modulate c-Fos Expression in Retinal Cell Cultures.

G.M. SEIGEL. Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

An E1A-immortalized retinal cell culture suitable for long-term *in vitro* studies was established in this laboratory and designated E1A-NR.3 [Seigel, submitted]. Expression of both E1A and c-Fos proteins was induced upon E1A-immortalization (neither protein was detected in primary, uninfected retinal cells). Immortalized retinal cells were maintained in DMEM, but demonstrated the capacity for extensive photoreceptor differentiation within 24 hours of treatment with depolarizing medium containing 30mM KCl [Seigel and Fideli, Investigative Ophthalmol. & Vis. Sci. abstract, submitted]. To investigate early events in the photoreceptor differentiation pathway, the kinetics of c-Fos immediate early protein expression were compared with E1A expression during the first 8 hours of treatment in depolarizing medium. Western immunoblot analysis revealed that E1A protein bands remained stable in intensity throughout the 8 hour course of depolarizing treatment. In contrast, c-Fos protein expression became undetectable within fifteen minutes of depolarization. Lack of c-Fos immunodetection could be due to protein degradation in conjunction with the short half-life of the c-Fos protein, or possibly a rapid conformational change rendering c-Fos antigenically unrecognizable. In either case, it appears that c-Fos protein: 1) is expressed as a result of the E1A-immortalization process itself and 2) is rendered undetectable as an immediate early event in response to depolarization. Thus, c-Fos expression and its transcriptional consequences may hold the key to understanding the growth and differentiation capacity of E1A-immortalized retinal cells *in vitro*.

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P-1001

Germline transformation of maize following particle bombardment of meristems. M. ROSS, K. Lowe, B.Bowen, D.Tomes, G.Hoerster, L. Church, L. Tagliani, D. Bond, D. Pierce, and W. Gordon-Kamm. Pioneer Hi-Bred International Inc. Johnston IA 50131

Chimeric plants were obtained at high frequencies from particle gun delivery into maize apical meristems. The developing meristem of the early coleoptilar stage embryo was an easily accessible and consistent target for transformation across a wide variety of genotypes. Bombardment of meristems followed by direct germination with or without selective pressure resulted in mericlinal or sectoral chimeras. Plants with large sectors extending into the ear and tassel were produced, however, transmission of the transgenes through the germ line was not observed. To enlarge and stabilize sectors, a shoot multiplication step can be used with selection. Axillary buds producing stable periclinal and possibly homogeneously transformed plants were identified and passed transgenes to progeny. Mendelian inheritance of the transgenes was confirmed by protein and molecular analyses in T1 and T2 progeny. These methods were used successfully on both hybrid and elite genotypes.

P-1002

Microprojectile DNA Delivery to Orchardgrass Leaf Cells [1]P.D. DENCHEV, [2] D. Songstad and [1]B.V. Conger. [1]Department of Plant and Soil Science, University of Tennessee, Knoxville, TN 37901, [2]Pioneer Hi-Bred Int'l., Johnston, IA 50131.

The leaf culture system in *Dactylis glomerata* L. (orchardgrass) in which somatic embryos initiate and develop from single mesophyll cells is an attractive system for gene transfer experiments. Tillers were selected from greenhouse grown plants and leaf segments were plated on SH medium with 30µM dicamba. Tungsten particles were coated with DNA plasmids containing the ***bar*** gene, and the ***uidA*** gene. Both genes were under control of either the **CaMV 35S** or maize ubiquitin ***Ubi*-1** promoter. Microprojectile bombardment was conducted with a PIG. The effect of culture time before bombardment, concentration of sucrose in the medium, distance from the syringe filter to the target tissue, and the two promoters were studied on transient expression of the GUS gene. The highest number of GUS spots was detected when leaves were precultured for 48h before bombardment. Culture for 72 or 96h decreased the number of spots. When leaf tissues were cultured for 4h on medium containing 15% sucrose before bombardment, GUS expression was enhanced 5-fold. The most spots were obtained when the leaf segments were placed 18.0 cm from the syringe filter. ***Ubi*-1** gave 2.3 times more GUS spots than **35S** and the spots were larger and more intensely stained. Also, somatic embryos induced directly from mesophyll bombarded cells stained blue after treatment with X-gluc for 24h at 37°C. More than 60 putative transformed plants resistant to Basta were regenerated on medium containing 1.5% bialaphos. Six of these plants showed no reaction when leaves were brushed with 0.01% Basta indicating resistance to the herbicide. Somatic embryos were produced from the tillers of these T1 plants on the medium containing 1.5% bialaphos and they also expressed GUS after treatment with X-gluc.

P-1003

Transformed Progeny via Particle Bombardment of Embryogenic *Cucumis melo* 'Eden Gem' Cotyledons. D.J. GRAY[1], E. Hiebert[2] C.M. Lin[2], K.T. Kelley[1], M.E. Compton[1] and V.P. Gaba[3]. [1]Central Florida Research and Education Center, University of Florida, 5336 University Ave., Leesburg, FL 34748, USA, [2]Dept. of Plant Pathology, University of Florida, POB 110680, Gainesville, FL 32611-0680, USA and [3] Virology Dept., Institute of Plant Protection, ARO, Volcani Center, Bet Dagan 50250, Israel.

'Eden Gem' is a green-fleshed, netted melon, with high embryogenic potential from cultured cotyledonary explants. Pretreated explants were bombarded with plasmid-coated microparticles, using a modified particle inflow-type gun. Transient expression of the ß-glucuronidase gene in plasmid pBI221 coated onto tungsten or gold microparticles was used to optimize bombardment parameters. Thereafter, plasmid pKYLX80 (obtained from Dr. A.G. Hunt, Univ. Kentucky) was utilized, which contains a doubled, CaMV 35S promoter, the NPTII gene for kanamycin (kan) resistance and was further modified by insertion of the coat protein gene of PRSV-W (also known as watermelon mosaic virus 1). Bombarded explants (120) placed on medium containing kan eventually became bleached; however, 443 green somatic embryos were produced, of which 25 produced shoots and roots when placed directly on kan medium. Five of the R_0 plants established in the greenhouse were Southern dot blot positive and four showed random insertion of the NPTII gene when subjected to Southern hybridization. Three of the R_0 plants were selfed and 72 seedlings from each were screened for NPTII expression by ELISA. Four seedlings from one R_0 plant and three from each of the other two were NPTII positive. PCR analysis of these ten R_1 plants showed that three contained the NPTII gene.

P-1004

Stable Genetic Transformation of Grapevine: Efficiency of Insertion of a Marker Gene in a R_0 Population. L. MARTINELLI and G. Mandolino. Laboratorio di Biotecnologie, Istituto Agrario, I-38010 San Michele all'Adige (TN), Italy.

A method for genetic transformation of grape based upon the infection of single somatic embryos of Vitis rupestris S. with Agrobacterium tumefaciens was set up in our laboratory, as already described (Martinelli and Mandolino, 1994, TAG 88:621-628). With this strategy the pBI121 cassette containing the GUS (ß-glucuronidase) gene has ben transferred; efficient transformation of the somatic embryos was achieved, and normal plantlets were regenerated and transferred to the greenhouse conditions.

Genomic DNA was extracted from a population of 8-10 cm tall plantlets, each deriving from different transformation and regeneration events, and collected both from in vitro micropropagated and in greenhouse acclimated plants. Eight independently transformed lines were tested, and in each line at least four individual plantlets deriving from different regeneration events were examined regarding gene insertion and position in the genome.

Out of 47 plants tested, 43 clearly showed the GUS gene in the inserted form, corresponding of a 91% efficiency of stable inserted gene. The copy number variation among the transformants was approximately estimated by probing a dot- blot filter with 5 µg of genomic DNA of the transformants with the ^{32}P-labelled GUS gene, and directly reading the filter- bound radioactivity with a beta counter.

Interesting observations were also collected on the position of the inserted gene into the genomes of the population tested.

P-1005

Production of Fertile Transgenic Peanut Plants Using *Agrobacterium tumefaciens*, and the Expression and Inheritance of Foreign Genes in Transgenic Peanut Plants. M. Cheng[1], A. Xing[1], Z. Li[1], R.L. Jarret[2], and J.W. Demski[1]. [1]Department of Plant Pathology, Georgia Station, University of Georgia; [2]USDA-ARS Regional Plant Introduction Station, Georgia Station, 1109 Experiment Street, Griffin, GA 30223, USA

Fertile transgenic peanut (*Arachis hypogaea* L. cv. New Mexico Valencia A) plants have been produced using an *Agrobacterium*-mediated transformation system. Peanut leaf sections harvested from in vitro grown seedlings were inoculated with *Agrobacterium tumefaciens* strain EHA105 harboring the binary vector pBI121 containing the gene for the scorable marker β-glucuronidase (GUS) and the selectable marker neomycin phosphotransferase II (NPTII). Leaf sections were cultured on shoot regeneration medium containing kanamycin. Transgenic shoots were identified by screening for GUS activity. Approximately 10% of the shoots regenerated on selection medium were GUS+. Five independent lines of fertile transgenic peanut have been obtained to date; a frequency of 3/1000 leaf explants inoculated. On average, 240d were required between seed germination for explant preparation and the production of T_0 seeds. Molecular analysis of T_0 and T_1 transgenic plants confirmed the stable integration of the foreign genes into the plant genome. The transmission of the GUS gene to the T_1 and T_2 generations verified that germline transformation had been achieved. The expression and inheritance of transgenes in transgenic peanut progeny will be presented.

P-1006

Genetic Engineering of Peanut (*Arachis hypogaea* L.). KIRAN K. SHARMA and J.P. Moss. Cellular and Molecular Biology Division, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, INDIA.

A tissue culture method was developed to obtain high frequency regeneration of adventitious shoot buds from cotyledon explants of peanut (*Arachis hypogaea* L.). Under optimum culture conditions, 90% of the explants produced multiple shoot buds that could be elongated, rooted and subsequently transplanted to the glasshouse with 100% success. This system was then employed for genetic transformations using *Agrobacterium tumefaciens* strains containing either neomycin phosphotransferase (npt II) and ß-glucuronidase (GUS-Intron) or hygromycin phosphotransferase (hpt) and GUS-Intron genes on binary plasmids. Freshly excised cotyledon explants from pre-soaked seeds were co-cultivated with the *Agrobacterium* suspension for 72 hours and maintained on the shoot regeneration medium containing appropriate antibiotics until at least 70% of the explants had produced multiple shoot buds. The shoots were elongated and rooted under selection pressure comprising of either 150 mg L^{-1} kanamycin (for npt II) or 25 mg L^{-1} hygromycin (for hpt). Following antibiotic selection, leaves and petioles from the putative transformants were tested histochemically for GUS gene, where at least 20% of the selected shoots produced a positive reaction. Molecular characterization of the marker genes based on PCR amplification and Southern blot hybridization of the genomic DNA from the putative transformants and their progeny showed consistent positive results confirming presence of the introduced genes. The in vitro formed plants exhibited normal growth habit, produced fertile flowers, and set viable seeds. Genetic analysis of the introduced genes in the putative transformants is being carried out. Detailed results on the in vitro technique and transformation aspects will be presented. Technical problems encountered with this system will be discussed.

P-1007

Accumulation of the 10 kD and 15 kD Zeins in Transgenic Plants. S. BAGGA, F. Rodriguez, N. Klypina, J.D. Kemp, and C. Sengupta-Gopalan. Plant Genetic Engineering Laboratory (SB, JDK, NK) and Department of Agronomy and Horticulture (SB, FR, CSG), New Mexico State University, Las Cruces, NM 88003-0003.

Legumes in general are deficient in S-amino acids. Our long term goal is to balance the amino acid composition of forage legumes by engineering genes encoding the proteins with high methionine content. The 10 kD and 15 kD zein proteins have high methionine content and are among the major seed storage proteins of maize. They are synthesized on the RER and are stored in the ER derived protein bodies in maize endosperm. Because of the high methionine content, the 10 kD and 15 kD zein genes are ideal to introduce into plants deficient in S-amino acids. However, it is crucial in our engineering efforts to determine whether the 10 kD and 15 kD zein proteins are stable in the various tissues of various transgenic plants. Toward this end, we have introduced the 10 kD and 15 kD zein genes behind the 35S Cauliflower Mosaic Virus promoter into tobacco, *Lotus japonicus* and alfalfa. Our results demonstrate that both the 10 kD and 15 kD zein proteins are stable in both the seeds and nonseed tissues of transgenic tobacco and *Lotus japonicus*; analysis of transgenic alfalfa plants are in progress. Data on the accumulation and storage patterns as well as the stability of the two proteins in transgenic plants will be presented.

P-1008

Cloning and Expression of Rice Tungro Spherical Virus Proteins and Introducing Genes into Rice Tissue. YITANG YAN, T.M. Burns, J.W. Davies and R. Hull. John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK.

Rice tungro disease, the most severe viral disease of rice in South and Southeast Asia is caused by a complex of Rice Tungro Spherical Virus (RTSV) and Rice Tungro Bacilliform Virus (RTBV). RTSV provides essential elements for leafhopper transmission and RTBV contributes most of the symptoms. Because of the indispensable role of RTSV in rice tungro disease, it should be possible to control this devastating viral disease by engineering rice cultivars with resistance to RTSV. RTSV has been classified as a member of the *Sequiviridae* family and has a genome of a polyadenylated single-stranded positive-sense RNA of about 12 Kb including one large ORF and two small ORFs. The large ORF encodes a polyprotein which is processed to functional proteins including coat proteins (CP1,CP2,CP3) and uncharacterised proteins both upstream (P1 gene) and downstream of the CP region. Individual CP proteins have been separately expressed in *E.coli*, purified and polyclonal antisera raised. Recently we have cloned and expressed P1 gene in *E.coli*. In order to produce transgenic rice plants with high level of viral resistance, we positioned individual RTSV CP genes downstream of the maize ubiquitin-1 5' regulatory sequence. The resultant constructs were used to transform rice plants. Currently a plasmid conferring resistance to hygromycin and bialaphos, and expression of GUS are being used to optimize rice transformation with mature embryos. The bombarded mature embryos exhibited high level of transient GUS activity. Two days after shooting, the embryos were subjected to 30–50 µg/ml hygromycin B selection. White resistant calli emerged from selected embryos two weeks later. Resistant calli continued to grow and appeared embryogenic upon transfer to fresh selection medium. We are using hygromycin resistance gene as a selectable marker to produce transgenic rice plants expressing RTSV CP gene.

P-1009

In vitro Plant Regeneration and Advanced Micropropagation Methods for Pineapple.

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We have developed an efficient and cost-effective method for commercial micropropagation of Smooth Cayenne pineapple. In vitro shoots were used as starting materials. Either longitudinal sections of the shoots or leaf bases were used as the explants to regenerate shoots. These explants allow the axillary meristems, which usually remain quiescent during shoot multiplication, to regenerate new shoots. Additional multiplication was achieved inside a 10-liter Nalgene vessel with shoots immersed in liquid medium for only 6 min every hour (periodic immersion bioreactor, PIB). The shoots were then induced to form roots and transferred to soil. Using the above regeneration method and the micropropagation in PIB, 6-8000 plants were produced from every three initial shoots within 4-5 months. The clonal fidelity of propagated plants is being tested in Costa Rican and Indonesian pineapple farms.

P-1010

Laser-Assisted *in vitro* Biology.

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Laser light, applied to the study of a variety of *in vitro* biological systems, is used to manipulate, fuse, purify and dissect biological samples at the cellular and sub-cellular level. In practice, when laser beams are focused through the objective lens of a microscope, they converge to form optical tools at the focal point for the lasers. Light in the UV or visible range of the spectrum forms an optical scalpel. Near IR wavelengths produce an optical trap. Such lasers and a high-precision, motorized microscope stage capable of motion control down to the micrometer level, transform microscopes into simple, integrated systems. Manipulations using light offer unique capabilities such as unrivaled sterile conditions.

Optical scalpel (*LaserScissors*™) and optical trapping (*LaserTweezers*™) technologies are presented. Applications are demonstrated in several fields of biology, including mammalian and plant cells, microorganisms and organelles. Laser microdissection of nerve cells is used to study signal transduction and cell response to injury. Microdissection and sorting of metaphase chromosomes is suggested for establishing specific genomic libraries for both plant and animal species. Laser puncture of the plant cell wall is proposed as an approach for transfection. Cellular interactions are promoted between natural killer cells and cancer cells. Single cells are purified, such as human lymphocytes (e.g. for the production of monoclonal antibodies). The relative positions of organelles within an intact living cell are modified using optical trapping. Several laser-assisted reproduction biology procedures are described for mammalian species (*in vitro* fertilization, embryo assisted hatching, preimplantation analysis, sperm capture).

P-1011

In Vitro Collection (IVC) -- Effects of technique and media on sterility and growth of cultures.

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In vitro collection (IVC) techniques were used to initiate cultures from over 120 species of plants from Trinidad and the Cincinnati area. Alcohol-sterilized tissues were collected using needle collection, leaf punch and other techniques. Needle collections from stems showed less contamination than leaf discs (52% and 9% clean-appearing, respectively), although Benlate decreased contamination rates from leaf discs (29% clean-appearing). Resterilizing contaminated tissues in the laboratory was successful in some cases. Callus growth was observed from approximately 25% of species collected. Collections from 12 Trinidadian species established clean-appearing callus lines. Several have regenerated shoots, and regenerated plants of *Hippobroma longiflora*, are being returned to Trinidad. All IVC callus lines are also being cryopreserved. Although a number of refinements are needed with regard to improved sterility, control of browning, and growth media for certain species, these results demonstrate that IVC can be used as an important component of "living collection" for the collection, growth and preservation of both temperate and tropical rare or endangered species, and could be used to provide frozen collections of species where seed storage is impractical or impossible.

P-1012

Regeneration of Whole Plants of Arachis hypogea L. from the Shoot Apex.

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Arachis hypogea L., peanut, has proven to be a difficult species to transform using Agrobacterium tumefaciens due to species-strain specificity and limited explant regeneration. The use of meristematic tissue as an explant source could increase the chance of A. tumefaciens infection and optimize regeneration. A method of regenerating whole plants from the shoot apex, which is defined as the apical dome plus one leaf primordia, of two cultivars is described. Seed of A. hypogea L., cultivars Florunner and Tamspan 90, were germinated in sterile conditions and the shoot apex excised from the primary shoot and the two lateral buds. The experimental design was two treatments, with and without an antibiotic in the media, having ten seed (thirty explants) per treatment, and the tests were repeated three times. After a five to seven week period on shoot apex medium the shoots were moved to a rooting medium containing one-half strength Murashige and Skoog salts and 1 mg/L napthelene acetic acid. At four to five weeks roots usually formed, and the plantlets could be transferred to soil. Preliminary data show 55% of Florunner explants formed roots after six weeks on rooting medium regardless of exposure to antibiotic. Root development continued to occur on explants left on rooting medium for three or more months. All Florunner plants that were rooted in culture and moved to soil have survived to maturity and are of normal phenotype. Similar experiments on Tamspan 90 are in progress and results will be presented.

P-1013

Micropropagation of Birch (*Betula pendula* Roth.) cv. Purple Rain from Shoot Tip and Bud Explants. K. PRUSKI and M. Younus, Alberta Tree Nursery and Horticulture Centre, R.R. 6, Edmonton, Alberta T5B 4K3.

Red leaved birch can be a valuable addition to the limited number of ornamental species grown in the harsh climate of the Canadian prairies. To develop an efficient system of mass multiplication of this species via tissue culture we tested the effect of media composition on shoot production and plantlet quality. Cultures were initiated from auxiliary bud and shoot tip explants on MS and WPM media (agar 6g/l) supplemented with vitamins and various BAP and IAA concentrations and grown at 24±2°C with 16h photoperiod, 50 $\mu Em^{-2}s^{-1}$ fluorescent light. Both, MS and WPM media were suitable for culture growth. The best shoot proliferation was observed at 1 mgl^{-1} of BAP (ratio 1:4 in 3 wks). At higher BAP levels shoots were shorter and more callus developed at the base of the cultures. This callus can be used for plant regeneration, however, few plantlets produced this way developed green leaves. Shoot tip explants grew faster and produced longer shoots than bud explants. All plantlets grown through shoot tip or bud cultures retained their purple leaf characteristic. IAA at 0.5 mgl^{-1} was the best concentration for rooting. Rooted plantlets were acclimatized for 4 wks on the greenhouse bench (23/18°C day/night temp.). Plants were field planted in spring 1992 and have shown an excellent survival rate so far.

P-1014

Genetic Stability of Micropropagated Strawberries. KUMAR, M.B., Dept. of Horticulture, Oregon State University, Corvallis, OR 97331, Barker, R.E., USDA-ARS (NFSPRC), 3450 SW Campus Way, Corvallis, OR 97331, and Reed, B.M., USDA-ARS (NCGR), 33447 Peoria Road, Corvallis, OR 97333-2521

Random Amplified Polymorphic DNA (RAPD) markers were used to determine if cold storage or supraoptimal levels of N6-benzyladenine (BA) in the culture medium caused genetic changes in micropropagated strawberries. Micropropagated 'Pocahontas' plantlets stored at 4°C for over four years were compared with pot grown mother plants. Ground leaf samples were divided into two subsamples and DNA extracted. Over one hundred Operon primers were screened initially for suitability in terms of reproducible deep bands and 30 primers were chosen for DNA amplification. Band profiles among samples were monomorphic for 29 of the 30 primers. The one primer (F) producing polymorphism was examined further on five cloned samples. Five explants were initiated into tissue culture, multiplied and later grown on medium with two different BA concentrations for seven months. Randomly chosen samples were screened for polymorphic amplification profiles using the same 30 primers. Two primers, one of which was F, produced polymorphic DNA amplification profiles. The other primers yielded monomorphic DNA amplification profiles. Except for the F primer, no evidence of mutations was seen in over 200 loci. No clear pattern matching polymorphism from F could be correlated with explant or treatment. One hundred plantlets from each treatment were planted for phenotype observation.

P-1015

Antiauxin Effects on Morphogenesis in Cultures of the Endangered Cactus *Aztekium ritteri*. I. REYES, J.F. Hubstenberger, and G.C. Phillips. Department of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88003-0003.

Many cacti are threatened with extinction due to destruction of their habitat, their collection from the wild, and their slow rates of reproduction. In vitro methods of culture and propagation have been investigated as a means to conserve endangered cacti. *Aztekium ritteri*, an endangered species native to Mexico, is slow to propagate either by conventional means or by micropropagation. Previous research suggested that in vitro cultures of cacti generally overproduce auxin or auxin-like growth regulators, resulting in excessive proliferation of callus at the expense of shoot development. This study was undertaken to evaluate whether antiauxin treatments could suppress callus proliferation and improve shoot morphogenesis in cultures of *A. ritteri*. Three antiauxins having different modes of action were tested: 2-(*p*-chlorophenoxy)-2-methylproprionic acid (CA, 0.02-2.0 mg/L), 2,3,5-triiodobenzoic acid (TIBA, 0.05-5.0 mg/L), and maleic hydrazide (MH, 0.1-10.0 mg/L). Short-term treatment (30 d) with these antiauxins had negligible effects on callus proliferation or shoot formation. Long-term treatment (>90 d) using high concentrations of the antiauxins resulted in suppression of callus proliferation. Long-term treatment using moderate concentrations of the antiauxins, especially CA, resulted in improved rates of axillary branching and improved the quality of shoot development. Histological analysis was performed on callus cultures to document the induction of de novo morphogenesis.

P-1016

Effect of Kinetin and BA on the In Vitro Culture of Potato Nodal Explants. M. A. BUSTAMANTE and S. Pérez. Department of Horticulture, UAAAN, Saltillo, Coahuila 25315, México.

The use of tissue culture techniques for potato tuber seed production has been established as an essential component in the cultivation of this important crop around the world. However, the micropropagation phase still has many factors which need to be studied to improve plant multiplication rates and plant quality. The objective of this work was to determine what concentration of Kinetin or BA, when combined with constant amounts of IBA and GA3, could induce the best responses in nodal explants. Shoot segments containing a single axillary bud and the leaf removed, were dissected from in vitro grown potato plants, and were plated on MS medium containing Kinetin or BA at 0.0, 0.5, 1.0, 1.5 or 2.0 mg/l + 0.2 mg/l IBA + 0.1 mg/l GA3. After 30 days we found that the % of explants with shoots, the lenght of shoots, the number of nodes on shoots and the % of explants with roots were superior with Kinetin than with BA, at all the concentrations tested; but in general, the best responses were observed with 0.5 mg/l of both cytokinins.

P-1017 Water Deficit Stress-Induced Gene Identification and Transformation in *Pinus*. M.A.D. Dias, P. Veeraragavan, J.H. Gould, and R.J. NEWTON. Department of Forest Science, Texas A&M University, College Station, TX 77843-2135

We have succeeded in isolating six cDNAs responding to water deficit stress from *Pinus taeda* (loblolly pine). They appear to be coding for proteins involved in changes in the cell wall. To understand their function more fully, we have initiated a plant transformation study employing both biolistic and *Agrobacterium*-based gene transfer methods. One cDNA clone (LP5), coding for a glycine-rich protein, has been cloned into several gene constructions containing the CaMV 35S promoter, *uida* (GUS) and *neo* (NPTII) genes for biolistic and *Agrobacterium*-mediated gene transfer. Transient and long-term expression of these proteins are under study in *P. taeda* tissue using the biolistic approach. Using the *A. tumefaciens* vector in the heterologous transformation models of tobacco and petunia, the expression of the LP5 construct is being studied. Inoculation of the *P. taeda* shoot apex with *A. tumefaciens* has produced shoots exhibiting a differential tissue specificity of GUS gene expression depending on the promoter used with *uidA*. The CaMV 35S promoter appears to drive expression in vascular tissue, whereas the larch Rubisco small subunit promoter drives expression in the apical meristem. Plantlets have been directly regenerated from inoculated shoots. Plant regeneration of various pine species has been achieved using embryogenesis, organogenesis, as well as the shoot apex approach. Nontransformed plantlets of *P. virginiana* (Virginia pine) and *P. taeda* derived through organogenesis have been field tested.

P-1018

Induction of Desiccation Tolerance by ABA and ABA-Analogs in Microspore-Embryos of Canola. X. Peng[1,2], D.C.W. BROWN[1,2,3], S.R. Abrams[4] E. Watson[2] and J.A. Webb[1]. [1]Carleton University, Ottawa; [2]Plant Research Centre, Agriculture and Agri-Food Canada, Ottawa, Canada, K1A 0C6, [4]Plant Biotechnology Institute, National Research Council, Saskatoon, Canada, S7N 0W9.

S-ABA (naturally occurring), R-ABA and a group of analogs were used to induce desiccation tolerance in microspore-derived embryos of *Brassica napus*. Results showed that, in inducing desiccation tolerance in microspore-derived embryos, the ABA structure requirement for biological activity is more flexible than in other systems. S-ABA at 10-100 µM was the most effective of the compounds tested in inducing desiccation tolerance, resulted in the best vigour after rehydration, and with concentrations applied below 0.1 µM, there was no observable effect. R-ABA was not as effective as S-ABA at concentrations of 10-100 µM, but was more persistent than S-ABA at concentrations of 0.1-1 µM. Three S-type analogs, P-89 (Dihydro ABA), P-82 (Dihydroacetylenic ABA) and P-63 (Dihydroacetylenic alcohol ABA) were equally effective in inducing desiccation tolerance, but the embryos needed an exposure to a 5-times-higher concentration to reach the same survival level of survival as with S-ABA. P-185, an enantiomer of P-82, was found to be as effective as P-82 at 50 µM. This is first time that P-185, a R-configuration analog, was shown to have biological activity. Two R-type enantiomers, P-59 and P-51, were found to be inactive. An ABA metabolite, phaseic acid, and ±*Trans-Trans* ABA did not induce desiccation tolerance. Applied ABA was taken up and metabolised during subsequent embryo incubation in culture. During the treatments with R-ABA and ABA-analogs, endogenous ABA increased a little suggesting that R-ABA and ABA-analogs were not converted to S-ABA, even when the concentration was high enough to be toxic to the embryos.

P-1019 **The Effects of Various Stresses on Protein Synthesis and mRNA in Soybean Cultures.** **P.S. KAHLON, S.M. Bhatti and Lan Qian. Cooperative Agricultural Research Program, Tennessee State University, Nashville, TN. 37209-1561.**

The development of soybean cultivars tolerant to the various environmental stresses is of great importance. In this study the hypothesis that stress of any kind affects the synthesis of proteins and mRNA in cells was tested. Cells were maintained in media containing the stressors NaCl, PEG or atrazine. The selected and nonselected cells were then transferred to media containing varying amounts of the above stress agents. The cells were harvested at different intervals of time and the growth rate of cells was determined by measuring fresh weight and packed cell volume. The total amount of protein was measured by SDS-PAGE. The mRNA was purified by Dynabead mRNA Direct Kit, and mRNA concentration was determined by Spectrophotometry. Results showed a decrease in growth rate with increasing concentrations of NaCl, PEG or atrazine. The average fresh weights in selected cells were higher than in nonselected cells. The total amount of protein increased in the 150 uM atrazine group and decreased in the 350 uM group after seven days. Analysis by gel electrophoresis showed that increased levels of 18- and 31- kD polypeptides occurred with increasing levels of atrazine. The mRNA concentrations increased in 150 and 250 uM atrazine. (supported by USDA/CSRS Project #TENX-9403-14-PS34)

P-1020

Methyl Jasmonate Induces Cathepsin D Inhibitor in Potato and Tomato Leaves. T.-H. ANNIE LIU and David J. Hannapel. Department of Horticulture, Iowa State University, Ames, IA 50011-1100.

Three plant species, tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum* cv. Superior) and a nontuber bearing species (*Solanum brevidens*), were tested for methyl jasmonate (MJ) induction at the RNA level using a petiole/leaf system. All of them show expression of the potato cathepsin D inhibitor cDNA p749 in leaves in response to wounding. It has been shown that MJ, a hormone-like, lipid-derived molecule, is considered to be a secondary messenger in wound signal transduction pathway and is a potent inducer of proteinase inhibitor II. The results of this study showed that the cathepsin D inhibitor is also induced by MJ. A dose response test was performed for MJ induction of p749 in the leaves of petiole/leaf cuttings. Induction was directly related to the concentration of MJ. For Superior and tomato, both had highest level of expression at 50 µM. The maximum expression level for *S. brevidens* was at 500 µM MJ. The induction of MJ was also dependent on the duration of exposure. Twelve hours of incubation were enough to give relatively abundant accumulation for all three species. There is no light/dark effect on MJ induction from 0 to 48 hours for either Superior or *S. brevidens*. But it has a slight decrease of expression using tomato leaf samples in the same treatment. Leaf samples of Superior and *S. brevidens* were also treated with 50 µM of GA either before or after MJ treatment to observe the interaction of these two hormones. GA had no effect on the MJ induction of p749 expression in either Superior or *S. brevidens*.

P-1021

Selection for Resistance to Inhibitors of Polyamine Biosynthetic Enzymes and for Adaptation to High Temperatures in Cotton Cell Cultures. R. Saavedra, G.D. Kuehn and G.C. PHILLIPS. Department of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM 88003-0003.

High temperatures during critical growth periods can seriously limit crop productivity. This research explored the relationship between enhanced biosynthesis of uncommon polyamines and mechanisms of tolerance to heat stress using cotton (*Gossypium hirsutum* L.) cell cultures. Selection was performed to develop cellular tolerance to known inhibitors of three biosynthetic enzymes in the polyamine pathway, which was predicted to result in overproduction of the targeted polyamines. Genotypes were chosen that were known to be tolerant or susceptible to heat stress conditions at the whole plant level. Under defined growth conditions, cell cultures expressed the heat stress tolerance trait in a manner that corresponded with known whole plant stress tolerance. Selected cell lines were recovered that exhibited stable adaptations to each of the inhibitors or to high temperature growth conditions. Many of the selected cell lines with adaptations to one of the inhibitors exhibited enhancements in the activity of one or more, in several cases all three, of the targeted enzymes. The activities of all three enzymes increased in response to cellular adaptation to high temperature alone, suggesting a relationship between adaptation to high temperature and uncommon polyamine biosynthesis. Attempts to regenerate plants from selected cell lines were not successful. (Supported by grants from the US Geological Survey and the NM Water Resources Research Institute)

P-1022

Callus Induction and Regeneration from Suspension Culture of Garlic, *Allium sativum*. J. MICHELE MYERS and Philipp W.Simon. USDA-ARS Vegetable Crops Research, Department of Horticulture, University of Wisconsin-Madison, Madison, WI 53706.

Garlic transformation has been hindered by the lack of an efficient callus induction system that can produce friable callus for suspension culture and subsequent regeneration. Root segments were evaluated as a possible explant source for a more efficient and rapid system. Garlic root segments from plants established in tissue culture were induced to produce callus by the addition of auxins and cytokinins. Three garlic lines were grown on a modified B-5 medium supplemented with either 2,4-D (1 mg/L), picloram (1 mg/L) + 2iP (0.1 mg/L) or no hormone. The root segments were evaluated for production of friable callus after 16 weeks and the best callus production occurred on root segments that were initially cultured on medium containing 2.4-D and then subcultured onto medium with picloram + 2iP. All callus was then transferred to medium supplemented with picloram + 2iP for two months. Embryogenic, friable callus from one line was subcultured for one month to suspension medium that consisted of modified B-5 + 2,4-D (1.0 mg/L). Garlic suspensions from different explant origin were then transferred to solid regeneration medium for three and a half months, subculturing monthly. Regeneration medium consisted of modified B-5 supplemented with picloram (0.1 mg/L) + BA (1.0 mg/L). The explant regeneration rate was 85.3% with an average of 6.3 shoots/explant. This system is very efficient in producing friable, regenerable callus and is being utilized in the development of a transformation system for garlic.

P-1023

Comparison of Different Methods of Regeneration for Soybean (*Glycine max* L.) from Mature Seeds and Immature Cotyledons. C.M. BAKER and C.D. Carter. Plant Science Department, Northern Plains Biostress Laboratory, Box 2140-C, South Dakota State University, Brookings, SD 57007

Regeneration in soybean (*Glycine max* L.) has been published using both immature cotyledons and mature seeds. To develop a regeneration system to be used in transformation studies, the method must be reproducible, with high efficiency, produce large numbers of structures per explant, and produce plants. Two systems for the regeneration of cv. 'Hendricks' have been developed. The first system is a multiple shoot system from mature seeds placed on a cytokinin-containing medium. The second system is an embryogenic system from immature cotyledons placed on an auxin-containing medium. Factors affecting these regeneration systems include plant growth regulator concentrations, types and length of exposure, photoperiod, carbohydrate concentration, explant age and type. Comparisons will be made between systems as to number of structures formed per explant, number of explants responding, and time to produce structures and plants. Studies are in progress on transformation using the particle inflow gun for the evulation of these systems for transient expression of GUS constructs.

P-1024

Utilization of Carbohydrates during Organogenesis of *Nicotiana tabacum* L. var. Burley 21
NEERAJ AHUJA and N. Dwight Camper
Department of Plant Pathology & Physiology, Clemson University, Clemson SC 29634-0377.

Changes in fresh weight, number of shoots, dry weights and carbohydrate utilization patterns were investigated during tissue culture of tobacco (*Nicotiana tabacum* L. var. Burley 21). Explants were grown on media with 0, 10, 20 and 30 gms/L sucrose for 28 days at standard culture conditions. The tissues did not vary greatly in fresh weight, dry weight and number of shoots but showed different levels of soluble carbohydrates during the culture period. Significantly higher sucrose, glucose and fructose levels were observed in tissues grown on media containing increased concentrations of sucrose. In all tissues, sucrose, glucose and fructose increased from day 21 to day 28. In tissues grown on 10 gms/L sucrose, both glucose and fructose levels dropped significantly between day 14 and day 21. In tissues grown on 20 gms/L sucrose, levels of fructose and sucrose in the tissue decreased significantly between day 14 and 21. For tissues grown on 30 gms/L sucrose, all the measured carbohydrates increased from day 14 to 21 of culture. This suggests varying patterns of carbohydrate utilization in tissues when grown on different initial sucrose levels.

P-1025

The Effect of Induction with 2,4-D versus NAA on the Origin, Histology and Normalcy of Pecan Somatic Embryos. Adriana A.P.M. Rodriguez and HAZEL Y. WETZSTEIN. Department of Horticulture, Plant Science Building, University of Georgia, Athens, GA 30602-7273.

The type of auxin used during induction has been reported to affect somatic embryo frequency, morphology and subsequent conversion into plantlets in a number of species. A clearer understanding of embryogenesis and the induction and developmental patterns associated with different auxins could be used to develop more efficient embryogenic systems. Using pecan (*Carya illinoinensis)* as a model, a histological and morphological comparison was made of somatic embryogenesis in cultures induced on medium with 2,4-dichlorophenoxyacetic acid (2,4-D) versus naphthaleneacetic acid (NAA) using light and scanning electron microscopy. Cotyledonary explants exhibited overall cell division and enlargement, followed by more intense cell division of localized regions. In cultures induced on NAA, proembryogenic protrusions were composed of homogeneous meristematic cells from subepidermal cell layers. In contrast, proembryogenic protrusions on cultures induced with 2,4-D originated from deeper cell layers, and were composed of meristematic globular proembryos dispersed with callus. Somatic embryos induced with NAA were generally single, with a discrete apical meristem. Those induced with 2,4-D were single or fused, and often fan-shaped with no distinct apical meristem; callus proliferation was marked. NAA-induced embryos exhibited higher germination/conversion rates than those induced on 2,4-D.

P-1026

Responses of *Sassafras albidum* (Nutt.) Nees Explants to IAA and 2,4-D in MS-Culture Media. C. E. Broderick. Department of Agriculture & Natural Resources, Delaware State University, Dover, DE 19901.

The sassafras plant is difficult to propagate, largely uncultivated, and a dioecious tree species which produces few viable seeds. It can however be a major source of safrole, used in the industrial synthesis of heliotropin and the insecticide piperonyl butoxide. The objective of the study, consequently, was to investigate the propagation of sassafras using tissue culture and micropropagation techniques. Modified MS media, with different concentrations of auxins and cytokinins, were used to grow and develop explants of leaf or stem tissues. Their responses to the auxins IAA and 2,4-D, and the cytokinins kinetin and benzyladenine, varied significantly, as measured in several experiments.

Both leaf and stem explant tissues grew into large calluses on 2,4-D media, at full strength or half strength MS salts. In response to IAA, leaf and explants readily callused, but the calluses were smaller than those with 2,4-D. Plant responses to auxins were also affected by the kind of cytokinin used. With 2,4-D, explants dedifferentiated and grew into calluses. Stem explants in 2,4-D/Kinetin media grew larger masses of callus than treatments of 2,4-D/BA. With IAA/Kinetin treatments, explants grew calluses whose tops remained pale green to white, but plantlets did not develop. With IAA/BA treatments, the period of callus growth was brief, followed by the appearance of one or a few meristematic centers, from which plantlets developed.

P-1027

Embryogenic Mango Suspension Cultures Challenged with Culture Filtrate of *Colletotrichum gloeosporioides* Show Enhanced Release of Extracellular Antifungal Proteins. S.JAYASANKAR and R.E.Litz. Tropical Research and Education Center, University of Florida, Homestead FL 33031

Anthracnose is a major disease of mango, caused by the fungus *Colletotrichum gloeosporioides* Penz. Most commercial mango cultivars are susceptible to this fungus. In an attempt to induce tolerance of this fungus, we recurrently selected embryogenic suspension cultures derived from the nucellus of two cultivars, 'Carabao' and 'Hindi' with 1) the culture filtrate and 2) a partially purified phytotoxic metabolite produced by the fungus. Dual culture studies showed a significant inhibition of fungal growth by the recurrently selected lines; however, conditioned agar medium containing 20% (w/v) of macerated recurrently selected embryogenic culture did not inhibit fungus growth, indicating the probable involvement of an extracellular compound in mycelial growth inhibition. There was enhanced extracellular production of some proteins as well as the appearance of newer polypeptides from recurrently selected lines. One isozyme of acidic chitinase was newly expressed by 'Hindi', while two other isozymes were induced in greater quantity in selected lines than in the control in both cultivars. A substantial increase in β-1,3-glucanase could also be detected in the selected lines by non denaturing PAGE. Our findings suggest that the enhanced secretion of pathogenesis-related proteins, either alone or in combination, confers tolerance of the anthracnose fungus in the selected lines.

P-1028

An *in vitro* Detection System for *Cornus florida* Calli Resistant to Toxic Metabolites of *Discula destructiva*. D. E. WEDGE[1], W. V. Baird[2], and F. H. Tainter[1] Departments of Forest Resources and Horticulture[2], Clemson University, Clemson, SC SC 29634-1003.

A reliable and quantitative *in vitro* detection system for toxin resistance in *Cornus florida* was established. Embryo-derived callus cultures which showed tolerance to culture filtrates from *Discula destructiva* were produced. A procedure combining ultrafiltration, lyophilization and rotary evaporation produced consistent isolation of low-molecular-weight compounds in a partially purified culture filtrate (PPCF). Callus cultures established on Murashige-Skoog-based agar medium were subjected to sublethal selection pressure using progressively higher concentrations of PPCF or potato-dextrose broth (control). Selection media were amended with either naphthaleneacetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D). Callus fresh weights were used to evaluate selected calli for resistance to toxic metabolites by challenge in a dose-response format with dose levels of 2.5, 5, 10 and 20% PPCF. Media amended with 2,4-D appeared to mask the effects of PPCF on dogwood calli which showed no significant difference between control calli and those selected with PPCF. Tolerance to PPCF by selected calli grown on NAA amended media was significantly increased by the selection process.

P-1029

Organogenesis and Expression of the Biosynthetic Pathways of Lipid Compounds in *Linum usitatissimum* and *Euphorbia characias*. A. Cunha and M. FERNANDES-FERREIRA. Departamento de Biologia, Universidade do Minho, 4719 Braga Codex, PORTUGAL.

Calli and shoot regeneration of *Linum usitatissimum* and *Euphorbia characias* were induced from shoot segments of axenically *in vitro* growing plantlets cultured on MS medium supplemented with zeatin (ZEA) and 2,4-dichlorophenoxyacetic acid (2,4-D) or indole-3-butyric acid (IBA). Either for *L. usitatissimum* or *E. characias*, hormonal supplementations with ZEA + 2,4-D were more efficient for induction and growth of calli, while supplementations with IBA + ZEA promoted better shoot regeneration. In the case of *L. usitatissimum*, the composition of the sterols fraction from regenerated shoots was similar to that of calli. On the contrary, organogenesis in *E. characias* was correlated with significant modifications of the expression of the sterols biosynthetic pathways which led to a greater diversity in the composition of the sterols fraction. Regenerated *in vitro* growing shoots of this species maintained the synthesis of the 4-demethyl sterols, usually produced by calli but not found in *in vivo E. characias* plants, and synthesized the 4,4-dimethyl sterols characteristics from their *in vivo* growing stems.

P-1030

Use of Clones in a Sugarbeet Improvement Program. L. PANELLA and C. Rivera Smith. USDA-ARS Crops Research Lab, Ft. Collins, CO 80526 and Summit Plant Laboratories, Inc., 2301 Research Blvd., Suite 106, Ft. Collins, CO 80526

Sugarbeet (*Beta vulgaris*, L.) is a cross-fertilizing, self-incompatible species. This results in each individual plant within a population having a unique genotype. Sugarbeets produce most of the sugar grown in this country; the crop is an economically important crop throughout the Northern Hemisphere. Plant Breeding and genetic improvement of this important crop, with traditional or new biotechnological methods, could be significantly enhanced through the increased availability of clonally propagated plants. A search of the literature reveals designs in which clonal material could and has proved valuable in a traditional breeding scheme. Sugarbeet clones can: 1) facilitate the production of hybrid seed for combining ability tests, 2) reduce the chances of producing chimeric tetraploids, and 3) minimize the space needed to maintain genotypes undergoing progeny (or clonal) testing. A theoretical examination of the use of clones in selection schemes shows that the potential increased efficiency of selection is proportional to the amount of genetic variation masked by the environmental variation present within the population. Clonal propagation also provides a means for maintaining important genotypes, such as mutants, haploids, parents for genetic studies, cell culture selections, and progeny from F_2 mapping populations. The availability of commercially produced sugarbeet clones could provide a powerful tool in the improvement of sugarbeet germplasm for public and private researchers who do not have access to the facilities necessary to produce clonal material on a research or production scale.

P-1031

Micropropagation of Cowpea (*Vigna unguiculata*) through Shoot Tip Multiplication. J.M. AL-KHAYRI, T.E. Morelock, and E.J. Anderson. Departments of Plant Pathology and Horticulture, University of Arkansas, Fayetteville, AR 72701.

Cowpea is a pulse crop that provides protein and other essential nutrients for human diets. It is also utilized as fodder and a cover crop. A limitation for applying biotechnology for the improvement of cowpea is the lack of a regeneration system. In this study, we induced shoot multiplication in cowpea, cv. Coronet, from shoot tip explants. Seeds, disinfected in 70% ethanol for 1 min. and 30% Clorox for 15 min., were germinated in vitro. Shoot tips, 5-mm long, were cultured on MS medium adjusted to pH 5.8 and augmented with (in mg L^{-1}) 100 inositol, 1 thiamine, 1 nicotinic acid, 1 pyridoxine, 2 glycine, 3% sucrose, and 0.8% agar. The medium also contained benzylaminopurine (BAP) or kinetin at 1, 2.5, or 5 mg L^{-1} combined with 2,4-dichlorophenoxyacetic acid (2,4-D) or naphthaleneacetic acid (NAA) at 0.01, 0.2, or 5 mg L^{-1}. Cultures were maintained at a 12-h photoperiod (40 $\mu Em^{-2}s^{-1}$) and 23±2C. Although to a variable extent, all cultures produced callus. Shoot tips cultured on kinetin-containing medium showed shoot growth that diminished as the concentration of auxin increased. Root formation, associated with kinetin treatments, was completely absent on BAP. Multiple buds and shoots developed on BAP-containing medium particularly with 0.01 and 0.1 mg L^{-1} NAA. Shoot tip may prove to be a useful explant for regeneration in cowpea.

P-1032

Micropropagation of agarita, *Berberis trifoliata*. F. MOLINAR, JR.[1], W.A. Mackay[2], M.M. Wall[1]. [1]New Mexico State University, Agronomy and Horticulture Department, Las Cruces, NM 88003. [2]Texas A&M University Research and Extension Center, 1380 A&M Circle, El Paso, TX 79927.

Actively growing shoots were collected from a mature agarita at the Texas A&M University Research and Extension Center in El Paso and successfully cultured on basal medium consisting of WPM salts and MS vitamins, $30g \cdot l^{-1}$ sucrose, 0.8% Phytagar supplemented with 11.1 μM BA. Experiments were conducted to optimize shoot proliferation. Cytokinins tested were BA, kinetin and TDZ. Other factors tested were subculture period, vessel volume, and age of cultures. The optimum proliferation conditions were WPM basal medium supplemented with 5.5 μM BA and a subculture period of 4 weeks. Culture age did not affect shoot proliferation. Rooting experiments tested the auxins NAA and IBA. Preliminary experiments with 1.0 μM NAA resulted in 100% rooting of shoots from cultures less than 6 months old. Rooting experiments with cultures maintained for several years indicate that rooting percentages decline dramatically. Currently, micropropagated plants are being evaluated in the field.

P-1033

Micropropagation of Banana Through Synseed Technology.
A.K. SUDHA VANI and G.M. Reddy, Centre for Plant Molecular Biology, Dept. of Genetics, Osmania University, Hyderabad-500007, India

Banana cultivation is carried routinely through suckers/corms and is limited by their availability. In the present study, suckers of dwarf cavendish besides local cultivars Amruthapani, Tellechakkerakeli, Karpura Chakkerakeli, Robusta and Yenugubontha were used. Trimmed suckers (4 cmss) were surface sterilised and each was cut into 16 longitudinal pieces and the explants were inoculated on to MS media with 6 mg/l BAp + 2 mg /l IAA + 20 mg/l adenine sulphate. Propagules (5 - 6) obtained after three weeks were thoroughly mixed with 2% sodium alginate prepared in MS basal salt solution and dropped into a solution of Cacl 2 2H20 (100 mM) by a pipette to obtain synseeds. Contamination was prevented using 250 mg/l Bavistin and 50 mg/l ampicillin. Out of various substrates tested for germination MS media gave 100% germination where as 66% germination was obtained on filter paper with MS nutrients followed by cotton 46% and vermiculite 12.6% suggesting that MS basal media is superior. Dwarf cavendish synseeds gave 100% germination. Yenugubontha did not exhibit any shoot initiationl suggesting varietal differences. Dwarf cavendish synseeds, cryyopreserved in liquid N2 for 30 days germinated with 33.0% viability. This offers a unique system for efficient propagation as secondary and tertiary propagules can be derived from 80 -100 primary propagules / sucker for continuous propagation.

P-1034

Inflorescence development from *in vitro* node cultures of switchgrass. K.S. ALEXANDROVA, P.D. Denchev, B.V. Conger.
Department of Plant and Soil Science, University of Tennessee, Knoxville, TN 37901-1071

The reproductive phase of perennial grasses follows enlargement and fast elongation of the stems. During the summer, in the greenhouse, this occurs in switchgrass (*Panicum virgatum* L.) at the 2 to 4 node stage. The top nodes, determined by the lack of a hollow space above the node of "Alamo" and "North Carolina 1-16" plants in that stage, were used as explants. They were surface sterilized, split longitudinally and plated on MS medium with 30 g/l maltose. BAP was added in concentrations of 5.0, 12.5, or 25.0 μM. At the time of plating the uppermost node contained a young inflorescence. The length of inflorescences in this study varied from 2 to 7 mm. Switchgrass inflorescences develop basipetally. The longest ones used already had well formed florets near the apex. After 2-5 weeks of culture, they were fully developed. Inflorescences developed on medium without BAP were between 5 and 9 cm long and the number of florets ranged between 50 and 200 per inflorescence. Those on medium with BAP were reduced in size (2 to 5 cm in length); the spikelets were directly attached to the main stem and there were between 200 and 700 florets per inflorescence. Florets were used as explants for regeneration experiments. Numerous somatic embryos were formed on MS medium with 22.5 μM 2,4-D and 5.0 μM BAP. They later developed into thousands of plantlets. This technique appears to be an effective and efficient method for obtaining plantlet regeneration in switchgrass tissue cultures.
Research supported by Martin Marietta Energy System Inc. under Contract No. 19X-SL129C.

P-1035

Use of an Acoustic Window in Ultrasonic Production of Nutrient Mist for Tissue Cultures. [1]M.J. CORRELL, [1]P.J. Weathers, [2]D. Walcerz, [2]J. Czarnecki, [2]M. Gibson, and [2]R. Owen. [1]Biology/Biotechnology and [2]Mechanical Engr. Depts. Worcester Polytechnic Institute, Worcester, MA 01609-2280.

Plants grown aeroponically have shown an increased biomass as compared to those grown in traditional shake flasks. Problems with current nozzle aeroponic systems are their expense and they produce large droplets of medium that are unable to pass through a dense culture of plant material, therefore creating areas of poor growth. Nutrient mist created by an ultrasonic transducer has droplet sizes 7- 10 μm allowing for deeper penetration of nutrients into the biomass. Our improved nutrient mist reactor separates the nutrient medium via an acoustic window and a layer of cooling water. The acoustic window allows the nutrient reservoir to be separated from electrical components while allowing ultrasonic energy to pass freely. This separation allows the reservoir to be autoclaved without damaging electrical components; it also allows for repair of electrical components during an experiment without compromising the sterility of the nutrient reservoir. The ultra-fine mist from the nutrient reservoir is pumped into a growth chamber. Our improved nutrient mist reactor is low cost and can be assembled in any laboratory. Results of growth using the nutrient mist generated by an ultrasonic transducer show a significant increase in biomass of cultures compared to those grown in shake flasks or on plates.

P-1036

IN VITRO PROPAGATION OF *LITSEA CUBEBA* (LOURS) PERS. (LAURACEAE)

A.A. MAQ, A. Wetten & P.D.S. Caligari

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A rapid clonal propagation system has been developed for the evergreen tree *Litsea cubeba*, extracts from the bark, leaf, root and fruit of which have been used in traditional Chinese medicine for the treatment of various ailments. The leaf is a primary food plant of the muga silkworm and the oil extract from the mature seed is reported to be effective in the control of broad bean weevil. A range of cytokinins have been investigated for multiple shoot induction with various explant sources (apical and axillary buds, leaf and petiole). 6-Benzylaminopurine (BA) and the apical bud proved to be superior for multiple shoot induction. In vitro rooting on growth regulator-free medium is possible and weaning to the glasshouse of over 150 plantlets was 90% successful.

P-1037

Cryopreservation of Arachis glabrata Benth. Shoot Tips by Vitrification. L.E. TOWILL. USDA-ARS, National Seed Storage Laboratory, 1111 S. Mason St., Fort Collins, CO 80523.

Vitrification has distinct advantages as a method for cryopreservation and is currently being examined for several vegetatively-propagated crops as a means of providing a low-maintenance back-up for materials held either in the field, greenhouse or in vitro. Some Arachis species are held as clones due to difficulties in flowering or in obtaining sufficient seed. I have utilized A. glabrata Benth. as a model system to explore vitrification of Arachis germplasm. Survival after liquid nitrogen (LN) exposure has been found using three common vitrification solutions and rapid cooling. Culture of excised shoot tips in 0.3 M sucrose enhanced survival after LN, but exposure of plants to cool temperatures for acclimation prior to excission did not. Loading of the shoot tips with dilute, permeating compounds (ethylene glycol, DMSO) did not reliably improve survival. Survival after LN varied considerably among experiments. The endogenous cellular contents (sugars, etc.) probably are important in attaining survival, and by inference I suggest that shoot tips from in vitro plants, even when grown under reproducible conditions, vary considerably in content of these essential constituents. I am currently comparing sugar contents in excised shoot tips after various preculture conditions.

P-1038

Velvetleaf (*Abutilon theophrasti*): The Effects of Thidiazuron on *in vitro* Culture of Seedlings and Seedling-derived Leaf and Hypocotyl Explants. C.A. Wiley and D.A. STEEN. Biology Department, Andrews University, Berrien Springs, MI 49104-0410.

Explants of velvetleaf, Abutilon theophrasti grown on solidified thidiazuron (TDZ) containing MS basal media exhibited marked hypocotyl expansion as measured by video image capture of silhouettes during incubation. Maximal expansion was seen at 0.1 to 10µM TDZ concentrations. Though not as striking, primary leaf explants expanded 30% more than the control when in 0.01 to 1µM TDZ. TDZ initiated and promoted callus development while preventing rhizogenesis in both leaf and hypocotyl explants. Seedlings which were germinated and grown on TDZ containing media had only 42% of the root length and 45% of the stem length of controls whereas the roots that did exist were severly swollen, enough to equal (1µM) or exceed (10µM) the root freshweight of controls. Callus development on intact seedlings was minimal. Induction of somatic embryogenesis was not observed in any of the cultures.

P-1039

Isolation of Rhodococcus Metabolites with Plant-Cell Regulation Activity by XAD-Adsorbent Resins .
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One of the major advantages in plant-microbial relationships is related to the induction of plant cell differentiation by means of specific metabolites with strong plant-growth regulation activity produced by microbial cells. In that context, an in vitro carrot tissue culture system was used in which the effects of Rhodococcus strains were monitored in cocultivation with the above described explants. The gathered data suggests that the bacteria were able to induce plant cell differentiation. A well defined cell differentiation in carrot's hypocotil and epycotil explants was induced by the microorganism. In here, a strong cell morphogenesis was observed since formation of shoots and roots were clearly developed. Also, centrifuging out bacterial cells, the supernatant showed regulatory activity over plant tissue culture. Consecuently, neutral resins were used to isolate the different microbial metabolites present in the supernatant which were correlated to cytokinins in nature. Such cytokinins were identified as kinetin and 6- - -Dimethyl allylaminopurine riboside (N^6 , Dimethyl allyladenosine) by TLC and GC analysis.

P-1040

Detection and Characterization of Bacterial Contaminants of Micropropagated Strawberry. PIYARAK TANPRASERT, Department of Horticulture, Oregon State University, Corvallis, OR 97331 and Barbara M. Reed, USDA/ARS National Clonal Germplasm Repository 33447 Peoria Road, Corvallis, OR 97333-2521.

Microbial contamination is an important problem in plant tissue culture. Early detection, identification and elimination of bacterial contamination may prevent the spread of contaminants and reduce losses. For early detection, three hundred runner explants from pot-grown strawberry plants were screened for contaminants using liquid Murashige and Skoog medium. Explant contaminants were 20% bacterial and 8% fungal. Bacterial contaminants from 70 explants were isolated, purified, and identified to genus using standard lab tests and the biolog test for carbon source utilization. The majority (55%) were fluorescent pseudomonads, 11% xanthomonads, some agrobacteria and other miscellaneous genera. Bacterial contaminants were 95% Gram negative and many were motile. Following this characterization, a variety of antibiotics (such as ampicillin, gentamicin sulfate, streptomycin sulfate, timentin, etc.) was tested to inhibit the bacterial growth. Minimal inhibitory and bactericidal concentrations were determined for selected bacterial isolates, and the response varied with genotype. Inhibition of bacterial growth by most antibiotics was best at pH 7.5. Phytotoxicity of the antibiotics varied with type, concentration and plant genotype.

P-1041

Bacterial Contaminants of *Corylus* In Vitro Cultures. BARBARA M. REED, Jessica Mentzer, Piyarak Tanprasert, Xiaoling Yu and Patricia Buckley. USDA/ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333-2521.

Persistent internal bacterial contamination is common with in vitro culture of *Corylus* (hazelnut). Plants may be lost during initiation as the bacteria overgrow the explant. Many *Corylus* explants survive with the bacteria present, however removing the bacteria improves the growth of most plants and decreases the risk of contaminating uninfected cultures. Bacteria were isolated from 14 infected *Corylus* cultures, purified, identified and treated with antibiotics. Bacteria from the cultures were checked for Gram character (+ or -), spore formation and growth in bacterial culture. Identification was based on carbon source utilization with the Biolog system. Isolates included both Gram positive and negative bacteria with representatives of *Agrobacterium sp.*, *Pseudomonas sp.*, and *Xanthomonas sp.* as well as other lesser known groups. Several antibiotics, including streptomycin, gentamicin and rifampicin, were screened for bactericidal action in liquid culture and phytotoxicity. The *Corylus* genotypes and the bacterial isolates varied in their response to the antibiotics. A combination of streptomycin (500μg/ml) and gentamicin (15μg/ml) was effective in eliminating bacteria from some hazelnut genotypes with only moderate phytotoxicity.

P-1042

Somatic Embryogenesis and Plantlet Regeneration in Rice Callus. S.SANGAM and P.B.Kavi Kishor,Department of Genetics, Osmania University, Hyderabad-7, India.

Low frequency plant regeneration and often loss of regeneration potential in callus cultures of Indica rice is a serious problem. To enhance and to revive plantlet regeneration frequency, rice callus was stressed on Linsmaier and Skoog's (LS) medium containing 9.05 mM 2,4-dichlorophenoxyacetic acid (2,4-D) plus NaCl or Na_2SO_4 or KCl or K_2SO_4 (100 mM each) or sorbitol or mannitol or erythritol (165 mM each) or polyethyleneglycol (PEG) (2%) from 5 to 60 days before subjecting it for plantlet differentiation. Regeneration was revived only in callus cultures grown on 2,4-D plus one of the above mentioned compounds for a minimum period of 20 days but not in callus grown on 2,4-D alone. However, 60 day treatment (3 subcultures in 2,4-D + one of the salts or sugar alcohols) was found to be optimum to generate higher frequency of potentially embryogenic, densely cytoplasmic, creamy white, compact callus and also subsequent plantlet differentiation (28-58%) upon transfer to a regeneration medium. Callus was also grown on LS medium fortified with 9.05 mM 2,4-D plus different concentrations (0 to 50 g/l) of PEG for 6 subcultures of 25 days each before testing its effect on regeneration. PEG at 2% level was found to be good for differentiation (41%). Cultures grown devoid of PEG could not produce any plantlets. Simple dehydration of callus on a filter paper and treatment of callus for 24 h in sorbitol, mannitol, sucrose, proline and abscisic acid (ABA) did not elicit any plantlet regeneration but callus grown on 10 mM proline for 20 days gave rise to plantlets with 35 to 43% frequencies.

P-1043

Growth regulator and genotype effects on somatic embryogenesis from sugarbeet callus. C.J. TSAI and J.W. Saunders. Dept. Crop and Soil Sciences and USDA-Agr. Res. Service Michigan State University, E. Lansing MI 48824

Opaque white somatic embryos up to 4 mm long were elicited from hormone autonomous sugarbeet (*Beta vulgaris* L.) callus within 5 weeks following plating of fresh suspension cultures grown on hormone free MS medium onto further hormone free MS medium. Suspension cultures had been initiated from approximately one month old leaf disc callus formed on MS + 1.0 mg/l 6-benzyladenine. The inclusion of 0.1 - 0.3mg/l abscisic acid in the plate out medium significantly increased the production of somatic embryos. Maximum average somatic embryo yield observed was 77 per ml of suspension plated out (minimum size for counting, 0.5 mm). Most somatic embryos developed into plantlets, often with betalain pigmentation on hypocotyls, after transfer onto hormone free MS medium. Genotype strongly influenced yield of somatic embryos.

P-1044

Increase in Somatic Embryogenesis from *Dactylis glomerata* L. Leaf Cultures by Silver Thiosulfate and Hypobaric Conditions. A.I. KUKLIN, C.E. Sams and B.V. Conger. Department of Plant and Soil Science, University of Tennessee, Knoxville, TN 37901.

In most cases increased ethylene has a negative effect on somatic embryogenesis. The objective of this study was to investigate whether inhibition of ethylene action by silver thiosulfate (STS) and ethylene depletion from culture vessels by reduced atmospheric pressure would affect somatic embryogenesis from orchardgrass leaf cultures and, if so, at which developmental stage. A 1.8 fold stimulation of somatic embryogenesis was observed with 5 μM STS. The number of regenerated plants obtained from leaves cultured for 5 or 30 d in a hypobaric chamber was significantly higher than that from control leaves. Studies indicate that hypobaric conditions delay senescence and force stomata to open in the darkness. At the experimental conditions employed in our experiment, stomata were closed and no differences in chlorophyll content between treatments and controls were observed. This indicated no effect of reduced atmospheric pressure on leaf senescence. However, it did increase ethylene depletion. Initial divisions occur in cells immediately subjacent to both adaxial and abaxial leaf surfaces 4 d after culture initiation. Our hypobaric experiment demonstrates that ethylene may be inhibitory to the initial cell divisions which lead to somatic embryogenesis.

P-1045

SOMATIC EMBRYOGENESIS IN PIGEON PEA (CAJANUS CAJAN.L)

Pigeon pea (Cajanus Cajan.L) is a high-Protein grain legume of the semi-arid tropics and caters to the Protein requirement of sub tropics. There is a limited information on Cajanus cajan tissue culture due to its recalcitrant nature. In recent years tissue culture techniques is used in crop improvement over conventional breeding methods. In the present study leaf callus derived from invitro raised seedling subjected to different 2,4-D (2,4-dichlorophenoxy acetic acid) concentration. Somatic embryo developement was achieved in single cell suspension culture. The appropriate media compositions and ontogency of somatic embryos will be discussed in detail.

P-1046

High Frequency Shoot Formation and Plant Regeneration from Mature Embryos of *Syzygium cuminii*. S.K. ROY and M.S.Islam, Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Multiple adventitious embryoids were developed from *in vitro* cultured mature embryo of *Syzygium cuminii*. Embryos of mature fruits were cultured on MS medium containing GA_3, BA and NAA. When the embryo axis was cultured on MS medium with BA + GA_3, multiple embryoids were induced within four weeks of culture. Optimum concentration of BA and GA_3 were 2.0 mg/l and 1.5 mg/l, respectively. The effect of GA_3 alone at 1-2.5 mg/l was found to elongate the embryo axis only. When the induced embryoids were subcultured with 1.0 mg/l BA + 0.5 mg/l NAA, they elongated to form shoots. The latter further elongated when subcultured on medium containing 10% coconut milk and 100 mg/l casein hydrolysate. Shoots and roots were not found to be induced simultaneously. The welldeveloped shoots were cultured in rooting medium. Within 3 weeks of transfer, 85% rooting was achieved on a medium consisting of ½ MS salts with 2.0 mg/l each of IBA and NAA. The rooted shoots were finally transferred onto soil.

P-1047

Effect of Silver Nitrate on Callus and Regeneration in Cotton Species. Z.-S. KE and J.McD. Stewart. Agronomy Department, University of Arkansas, Fayetteville, AR 72701

Silver nitrate ($AgNO_3$) inhibits ethylene action in plant tissues. Because ethylene may impact cultured tissue negatively, the effects of $AgNO_3$ on callus induction and on regeneration were studied using 8 genotypes involving 3 cotton species, *Gossypium herbaceum* (A1), *G. arboreum* (A2) and *G. hirsutum* (AD1). Coker 312, an AD1 cultivar, was the only genotype known to be embryogenic. Hypocotyls from 10 cm etiolated seedlings were cut into 0.5 cm segments and cultured on 50 ml/plate MS medium with 0.1 mg/l 2,4-D, 0.5 mg/l kinetin and 0.9% agar. Treatments were 0, 5 μM, 10 μM, 20 μM, 40 μM, 60 μM $AgNO_3$. Callus growth was measured as fresh weight (FW), dry weight, and cell number/250 mg FW. The effects of $AgNO_3$ on FW differed according to the genotype. It increased callus FW of the A1 genotypes but decreased that of AD1 cultivars. Cell number/mg FW of callus varied according to genotype and $AgNO_3$ concentration. All concentrations of $AgNO_3$ prevented browning of callus tissue. With $AgNO_3$, A1 and A2 calli remained green while AD1 calli remained light tan. All calli became brown with age in the absence of $AgNo_3$. Genotypes that were nonembryogenic in the absence of $AgNO_3$ did not become embryogenic in its presence. However, Coker 312 appeared to have enhanced embryogenesis with 20-40 μM $AgNO_3$. At these concentrations cell number/mg FW was maximum and approximately the same. At lower and higher concentrations both cell number and embryogenic potential were less.

P-1048

Asymmetric Somatic Hybridization via Protoplast Fusion in Peanuts. Z. Li[1], A. Xing[1], M. CHENG[1], R.L. Jarret[2], R.N. Pittman[2] and J.W. Demski[1]*. [1]Dept. of Plant Pathology, Georgia Station, Univ. of Georgia; [2]USDA-ARS Plant Genetic Resources Conservation Unit, Georgia Station, Griffin, GA 30223, USA

A protocol for protoplast fusion and plant regeneration in peanuts (*Arachis* spp.) has been developed. Conditions for efficient isolation of protoplasts from immature cotyledons of cultivated peanut (*A. hypogaea*) and young leaves of peanut-related species (*A. glabrada* and *A. digoi*), known to possess virus resistance, have been optimized. Up to $10x10^6$ and $2x10^6$ protoplasts per gram tissue were routinely obtained from the cultivated peanut and the wild peanut tissues, respectively. Asymmetric somatic hybridization was employed to fuse x-ray-irradiated donor protoplasts of peanut-related species with the recipient cultivated peanut protoplasts. An exposure dosage of 5 Krads of x-ray irradiation was sufficient to fragment donor cell chromosomes and inhibit protoplast colony formation. A frequency of up to 20% of effective fusion events was routinely achieved using a fusion solution containing 20% polyethylene glycol and 10 mM Ca_2Cl with a pH6.5. Fusion products were readily regenerated into callus colonies using our previously defined protoplast regeneration protocol. After transfer to a shooting medium containing TDZ, up to 88% of the callus colonies produced plantlets within a period of 3 months. Up to 200 putative cybrid plantlets have been obtained. These plantlets are being moved to the greenhouse for further cytological evaluation for chromosomal introgression.

P-1049

In Vitro Shoot Multiplication of Carnation Axillary Buds and Nodes. M.S. BRAR, J.M. Al-Khayri, and G.L. Klingaman. Departments of Horticulture and Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Carnation (*Dianthus caryophyllus* L.) is an important cut flower in the global market and accounts for a large percentage of the total sales through the Dutch auctions. Tissue culture provides an attractive means for its propagation. This study was conducted to study the response of nodes and isolated axillary buds of the cultivar Barlo II Nora to shoot multiplication treatments. Growth regulators tested were 1-naphthaleneacetic acid (NAA) at 0, 0.002, and 0.02 mg/L, combined with benzylaminopurine (BA) at 2.5, 5, 10, 15, and 20 mg/L supplemented to Gamborg's (B-5) basal salts. The medium, adjusted to pH 5.7, also contained 100 mg/L *myo*-inositol, 50 mg/L casein hydrolysate, 0.1 mg/L pantothenic acid, 1 mg/L nicotinic acid, 1 mg/L pyridoxine, 1 mg/L thiamine, 2 mg/L glycine, 30 g/L sucrose, and 8 g/L agar. The cultures were maintained at a 10-h photoperiod (40 $\mu Em^{-2}s^{-1}$) and $23\pm2C$. After 6 weeks, shoot length and shoot number per explant were determined. Shoot multiplication and shoot length were influenced by the concentration of the growth regulators. A medium containing 0.02 mg/L NAA and 5 mg/L BA appeared to induce a high rate of shoot multiplication. Axillary buds resulted in higher shoot multiplication than nodal explants. This study may contribute to the development of a micropropagation system specific for this carnation cultivar.

P-1050

The Effects of Ethylene and Ethylene Inhibitors on White Spruce Embryogenic Tissue Maintenance and Somatic Embryo Maturation. LISHENG KONG[1] & Edward C. Yeung[2]. [1]Dept of Biology, University of Saskatchewan, Saskatoon, Sask, Canada S7N OWO. [2]Dept of Biological Sciences, University of Calgary, Calgary, AB, Canada T2N 1N4

The influence of ethylene on embryogenic tissue maintenance and somatic embryo maturation of white spruce (Picea glauca) was studied through applications of ethephon, silver nitrate ($AgNO_3$), silver thiosulfate (STS), cobalt chloride ($CoCl_2$), and aminoethoxyvinylglycine (AVG) in either maintenance or maturation medium. Ethylene was produced by the embryogenic tissue during tissue maintenance and embryo maturation. $AgNO_3$, STS and $CoCl_2$ could suppress ethylene production to some extent while AVG was the most effective treatment. Though ethylene production corresponded to the growth of embryogenic tissue during maintenance, suppressing ethylene production by the inhibitors did not have a significant effect on tissue growth. Embryogenic capacity of the tissue could be prolonged in an older maintenance culture by application of AVG. In the maturation medium, the presence of AVG or $AgNO_3$ improved the quality of the embryo by reducing intercellular space formation in the shoot pole. $AgNO_3$ and $CoCl_2$ were effective to a much lesser extent than ABA in promoting embryo production. In the absence of ABA, AVG could not stimulate embryo formation. STS was harmful to tissue growth. Ethephon inhibited embryo production and enhanced intercellular space formation. Thus, inhibition of ethylene biosynthesis and action could have a positive effect on white spruce embryogenic tissue maintenance and somatic embryo maturation.

P-1051

Propagation of *Rauvolfia serpentina* by *In vitro* Shoot tip Culture - S.K. ROY, M. Z. Hossain & N. Alam. Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh.

Rauvolfia serpentina (Fam. Apocynaceae) is an important medicinal herb. Mass scale collection of this species from natural habitats is leading to a depletion of plant resources. For the conservation of this valuable genotype, we established a protocol for mass propagation. Shoot tips and lateral buds from field-grown plants were used as explants. When the explants were cultured on MS medium with 1.5 mg/l BA + 0.5 mg/l NAA multiple shoot buds formed. Subculture on the same nutrient medium gave higher number of shoots. When each of the regenerated shoots was excised and subcultured individually on the same nutrient medium, they also produced multiple shoots. Thus the shoots had to proliferate through ten subcultures with an average of 15 shoots per transfer. For rooting, shoots were excised from culture flasks and implanted individually on root induction medium consisting of half-strength MS salts supplemented with 1.0 mg/l each of IBA and IAA. Within 3-week of transfer, 100% rooting was achieved on this medium. They were transferred to a tray containing soil and covered with polyethylene sheet where 95% of the plantlets survived. After two weeks the regenerants were transferred to the field where 95% of the plantlets survived.

P-1052

Genotypic basis for multiple shoot induction from de-embryonated cotyledons of groundnut. SABITHA, A and G.M.REDDY, Centre for Plant Molecular Biology, Dept. of Genetics, Osmania University, Hyderabad-500 007, A.P., INDIA.

De-embryonated cotyledons from 16 genotypes of wild and cultivated groundnut were inoculated onto MS medium supplemented with 5.0 mg/l BAP + 0.5 mg/l IAA. Cotyledons turned green within 3 to 6 days and shoot buds appeared within 15 days of initiation. Though all the genotypes produced shoots, the frequency of multiple shoot induction was found to be genotype dependent (25 to 60%). The cultivated ICG 221, ICG 7827, ICG 1908, ICG 1697, ICG 2710, ICG 2448, ICG 476, ICG 799, ICGS 11, ICGS 44, ICG 2716, ICG 100028, ICG 100075 besides wild species A.duranensis, A.cordensis and A.monticola were used in the present study. ICGS 11 and ICGS 44 gave more number of shoots/cotyledon and high frequency of multiple shoot induction (66%). Shoots were induced with simultaneous production of callus in ICG 221 and ICG 476. The multiple shoot induction as well as number of shoots per cotyledon was low in ICG 100028 and ICG 100075 (36%). In wild species (A.duranensis, A.cordensis and A.monticola) the number of multiple shoots ranged from 3 to 6 and in general the frequency of multiple shoot induction was low (25-29%). Regenerated shoots upon transfering to MS medium, supplemented with 4.0 mg/l IAA + 0.5 mg/l K_R exhibited well developed root system. This method of direct shoot regeneration is useful in genetic transformation studies in groundnut.

P-1053

Induction of enhanced plant regeneration from callus cultures of some *indica* rice varieties. J.S.Sandhu*, M.S.Gill[1] and S.S.Gosal[2]. *Downing College, University of Cambridge, CB2 1DQ, UK, [1]Department of Plant Breeding, [2]Biotechnology Centre, Punjab Agricultural University, Ludhiana 141 004, India.

Callus cultures were established from mature seeds of four *indica* rice varieties viz. IR 54, Jaya, Pusa Basmati 1 and Basmati 370 by culturing on Murashige and Skoog medium. Maximum callus induction ranged from 68 per cent in Jaya to 94 per cent in IR 54 on MS medium supplemented with, 2,4-D (2.0 mg/l) + Kin(0.5 mg/l)+ sucrose (6% w/v) + mannitol (1% w/v). Addition of activated charcoal (2% w/v) in callus maintenance and multiplication medium enhanced the culture response. On transferring to the regeneration medium the nodular calli exhibited development of green islets and subsequently normal shoots. Best shoot regeneration occurred on MS medium supplemented with BAP (0.5 mg/l) + sucrose (4.5% w/v) + mannitol (1% w/v), which ranged from 6.5 per cent in Basmati 370 to 89 per cent in IR 54. Regeneration efficiency was found to decrease with the age of callus. Complete plantlets were obtained by transferring the separated shoots on rooting medium MS + IBA (1.0 mg/l) + sucrose (3% w/v). The tissue culture derived plants were grown in field to maturity and showed variation in morphological traits.

P-1054

In Vitro Regeneration and Protoplast Culture Studies in Mungbean(Vigna radiata (L.)Wilczek). D.TAMIL SELVI,N.M.Ramaswamy,S.Sukumar and S.R.Sree Rangasamy. Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamilnadu, India.

The in vitro response of three mungbean(Vigna radiata) cultivars(CO.3,CO.4,CO.5) were studied for callusing and regeneration using three explants viz., leaf,hypocotyl and seed in MS media supplemented with different hormones (2,4-D,BAP,Picloram) and with growth additives. These explants showed callus formation within 15 days. The callusing frequency of explants in MS medium with 10% palmyrah milk was high(92%). The calli were subcultured and alginate beaded in solid and liquid MS media supplemented with various levels of hormones for regeneration. The hypocotyl derived calli of one cultivar (CO.5) under low light in MS+BAP(2 mg/l) + kn(0.5 mg/l)+NAA(0.5 mg/l) showed regeneration. Protoplast isolation and culture studies with mesophyll and hypocotyl cells required 0.35 M (Mannitol) as optimum osmoticum. Viable protoplast yield was obtained from primary calli(20 days) of leaf and hypocotyl explant. The protoplast showed cell wall regeneration and division (21.7%) in V 47 medium + 2,4-D(2 mg/l). Our studies on recalcitrant mungbean indicate the possibility for applying tissue culture technique for its improvement.

P-1055

Factors affecting organogenesis and somatic embryogenesis in eggplant P. Sharma & M. V. Rajam. Dept. Genetics, Univ of Delhi, South Campus, Benito Juarez Road, New Delhi-110021, INDIA.

The relative importance of genotype, explant and their interactions for *in vitro* plant regeneration via organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.) was studied using hypocotyl, cotyledon and leaf explants of 4 Indian cultivars. Shoots could be obtained either on hormone free medium or 11.1 μM BA in combination with 2.9 μM IAA. While 32.2 μM NAA was found to be optimum for hypocotyl explants, 10.7 μM was optimum for leaves cotyledons. Genotype, explant and genotype explant interaction had highly significant effects on both morphogenic processes with genotype exerting maximum effect; Pusa Purple Long was the most responsive genotype. Among the explants, hypocotyls yielded the maximum number of shoots followed by cotyledons and leaves. The embryogenic response of leaves and cotyledons was, however, significantly higher than that of hypocotyl explants. Significant differences for morphogenetic potential were also observed in different regions of the hypocotyl. There was a basipetal gradient for organogenesis on hormone-amended medium; however, terminal hypocotyl segments yielded more shoots on hormone-free medium as well as somatic embryos, than the medial segments.

P-1056

In Vitro and In Vivo Multiplication of Virus-Free "Spunta" Potato. R.A.Shibli*, A.M.Abu-Ein and M.M.Ajlouni. Faculty of Agriculture, Jordan Univ. of Sci. & Tech. Irbid - Jordan.

Spunta potato microshoots (3.0 cm, 3-4 nodes) were subcultured on liquid MS media containing (0.0,0.5, 1.0,1.5 and 2.0 mg/l) benzyl adenine (BA) or kinetin (KI). Significant reduction in stem and internode length was shown with increasing BA and KI concentration. Benzyl adenine (up to 1.0 and 1.5 mg/l) resulted in increasing number of proliferated shoots and number of nodes per flask. Single node cuttings were rooted on solid media containing NAA, IBA, or IAA (at 0.0,0.5,1.0,1.5,2.0 mg/l, individually) with or without 1.0 mg/l GA_3. Microtuberization did not respond to the increase of BA or KI in liquid media while increasing sucrose concentration from 30 to 90 g/l gave a significant enhancement in number of produced microtubers and proliferated shoots. Cuttings (3.0 cm) from glasshouse grown mother stock were successfully rooted by treating them (basal 0.5 cm) with 1 g/l IBA + 0.5 g/l IAA for five seconds.

P-1057

Induction of Direct multiple Shoots from Cotyledons and Meristems of Gossypium Hirsutum L.

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Cotton, Gossypium hirsutum is economically important and prone to attack by many insect pests. The present work was carried out with ten Indian cultivars namely NCS 3, NHH 390, Srisailam, MCU5, Priya, NA 1325, PMC, DCH 32, Anjali and LRA. Multiple shoots were induced from meristems / cotyledons inoculated onto MS medium, B5 vitamins, 3% Glucose supplemented with 5 mg /l BAP, within 2 weeks. The no. of shoots per explants varied from 7 - 10 depending on cultivar. Highest number of multiple shoots (10) were indkuced in NCS 3. NCS 3 cultivar exhibited 90% of multiple shoots and other varied from 10-45 %. The regenerate were transferred to 5 mg / 1 IAA media, and roots were regenerated profusely within 2 weeks. Regenerated plants (30) were transferred to pots in the Net house. The present technique is being exploited in transformation studies with Bt gene constructs in developing Heleothis resistant types .

P-1058

Induction of Embryogenesis by Anther Culture of Pigeonpea. P. VIJAYAKUMARI & S. Narasimha Chary, Cytogenetics & Tissue Culture Laboratory, Dept. of Botany, Osmania University, Hyderabad 500 007, India.

Pigeonpea is considered to be an important legume of semi arid tropic areas. Plant regeneration may be possible from microspores via direct embryogenesis or organogenesis or embryogenesis from callus or from other parts of anther tissue. Light microscopy, scanning electron microscopy and histology were used for careful study of morphogenesis of microspore derived plants from anther culture on modified MS and N6 medium to show embryogenesis. We report that plantlets are obtained via embryogenesis from uninucleate microspores of anthers. Evidences for embryogensis from SEM included the formation of epidermis and suspensor like structures followed by initiation of apical meristem. Light microscopic studies revealed the different developmental stages of globular embryos from the microspores after 45 days. Histological studies showed 4,8,16,32 celled globular embryoids and late globular stages with a notch and suspensor cells attached. These embryoids developed further into complete plantlets when cultured in MS ½ strength medium, supplemented with NAA and BAP. Cytological studies revealed the haploid chromosome number. These regenerants obtained via embryogensis from microspores will be more useful for breeding programmes.

P-1059

Callus Induction, Plant Regeneration and Somatic Embryogenesis in Primary Trisomics (2n+1) of Indica Rice Oryza Sativa L. **NUZHAT FATIMA** and **S.Y. ANWAR**
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In our studies with mature embryo explant of control (2n) and primary trisomics (2n+1), LS medium with 2.0 mg/l 2,4-D + 2% Sucrose was used for Callus induction. For regeneration, modified LS medium containing 0.1mg/l IAA and 0.4 mg/l Kinetin + 2% Sucrose was used. Observations recorded on the callusing percentage and frequency of plantlet regeneration have shown that in general, mature embryos of different primary trisomics resulted in increased percentage of callusing (75%-100%) and plantlet regeneration (75%) compared to the control (10%). Studies on somatic embryogenesis with (2n) and (2n+1) explants have shown that in primary trisomics (2n+1), compact nodular calli with many somatic embryoids were observed, while in control friable callus with relatively less number of Somatic embryos were seen on LS medium containing 2.0 mg/l. 2,4-D + 0.5 mg/l Kinetin and 3% Sucrose.

Anatomical studies revealed the presence of globular shaped proembryoids/embryoids in almost all the embryogenic cultures of primary trisomic (2n+1) used in the present study.

In general, highly embryogenic calli were observed, when derived from (2n+1) embryo explants when compared to (2n) embryo explant.

Observations made in the present study clearly suggests that the presence of additional chromosome in the explant resulted in the increased frequency of callusing, plant regeneration besides, induction of Somatic embryogenesis and plantlet regeneration.

P-1060

PLANT REGENERATION FROM IMMATURE EMBRYOS OF 48 ELITE CIMMYT BREAD WHEATS

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Forty eight bread wheat (*Triticum aestivum* L.) released cultivars and elite advanced lines were evaluated for their ability to produce embryogenic callus using 3 different media. Basal N6 medium supplemented with dicamba (E1), MS medium containing 2,4-D (E3) or MS medium containing 2,4-D plus different amino acids (E5) were used for callus initiation and maintenance. Plant regeneration was achieved on basal MS medium with Indole-3-acetic acid (IAA) and 6-Benzylamino purine (BAP) and rooting on MS with 1-naphthaleneacetic acid (NAA).

Percentage regeneration varied widely with both genotype and initiation medium with values ranging from 2 to 94%. Plantlets produced per embryo ranged from 6 to 42. Sixteen of the genotypes showed a minimum of 50% regeneration on at least one of the media: two percent of the genotypes showed at least 50% regeneration on E1 medium; 6% on E3 and 28% on E5. After 4 subcultures, over a 16 week period, 41 genotypes (85%) lost their ability to regenerate plants and only 7 lines (15%) retained plant regeneration potential with reduced levels. E3 medium was the best for maintaining regeneration potential after four subcultures.

These results will form the basis for future efforts aimed at transforming CIMMYT bread wheat varieties

P-1061

Isolation of Fattyacid Desaturase Genes (Fad 2 and Fad 3) from Genomic Library of Arachis Hypogaea L.

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Arachis Hypogaea L. (Peanut) is an important legume rich in edible oils. The fatty acid desaturase genes (Fad 2 and Fad 3) are involved in enhancing the Polyunsaturated fatty acid (PUFA) content viz., linolenic acid which actually lessens cholesterol. Isolation and manipulation of these genes would nutritionally enrich peanut oil quality. Genomic library of peanut was developed in Lambda GEM 11 vector with a view to isolate and characterise the genomic colones of these genes. The vector DNA was partially filled in with dTTP and dCTP. The xho I arms were used in conjunction with Sau-3 AI digested peanut DNA which were partially filled in with dATP and dGTP. This approach eliminated the need for size fractionation of digested genoic DNA prior to ligation. The resulting library was amplified in LE 392 bacteria to yield packaging efficiencies as high as 1.5×10^{12} pfu/ml. Heterologous probes of Arabidopsis fad 2 and fad 3 are being used to screen the library to isolate the recombinant clones of desaturase genes for further characterisation.

P-1062

Molecular Studies on in vitro flowering in Arachis hypogaea L.

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Direct in vitro flowering in Arachis Hypogaea (Peanut), a day nutral oil seed legume, offers an efficient system to study the molecular basis in floral initiation. Deembryonated cotyledons of spanish bunch variety TMV 2 were inoculated onto Blaydes' medium with 6 Benzylaminopurine (BAP, 0.5 mg/l). About 8-24 flower buds were developed directly over a cotyledonary extension (2-3 cms) within 20 days of inoculation . Flower initiation was inhibited in the absence of or with excess BAP.

Histological and SEM studies of flower initiation suggested that the floral primordia were formed within six days of culture. Polypeptides of 25 kd, 18 kd and 10 kd were associated with flower initiation and the 10 kd polypeptide was observed only from day 2-4 during flower initiation. Molecular studies are in progress to identify the homologous gene of AGAMOUS (AG) of Arabidopsis, involved in floral meristem determinacy and floral organ identity. Studies with RNA blots during first six days of flower initiation in TMV 2, indicated that the gene homologue of AG is expressed within 3 days (72 hours) of initiation. The expression of this gene is being analysed in cultivars of peanut with varying frequency of in vitro flowering from 0% to 22.5% in CGC 5 and TMV 2 respectively.

P-1063

Selection for Improved Agronomic Value in Pigeonpea Somaclones Regenerated from Cotyledonary Explants.

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Tissue culture methods leading to somaclonal variation have been used to generate enhanced and heritable genetic variability. To select for high yielding and disease or insect resistant somaclones, we developed an efficient and high frequency regeneration from seedling cotyledons. The R1 plants, regenerated without any deliberate selection pressure in culture, showed floral alterations which were absent in their sexual progeny and are thus assumed to be epigenetic manifestations of tissue culture. The R2 plants segregated for flower colour, plant height, leaf shape and flowering habit. The statistical analysis of the R2 data on the various yield parameters like plant height, helicoverpa damage, 100 seed mass, biomass and harvest index has shown significant variations for these traits within the progeny populations as well as among the different R1 plants. The frequency of variant phenotypes within progenies arising from individual explants varied from 0.25 to 4.00. Chi-square analysis of R2 progenies showed that the variant traits segregated according to Mendelian ratios, and were heterozygous in R1. Thus tissue culture system of pigeonpea generated highly variant material. Based on the cluster analysis of the summary statistics, plants were selected for high yield, high seed mass, white seed coat colour and low helicoverpa damage. Molecular characterization of the selected plant progenies by RAPDs using polymerase chain reaction is underway. The preliminary results showed polymorphism for the traits under study.

P-1064

High frequency Callusing and Green Plant Regeneration from Anthers of Indica Rice

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Protocol for high frequency of callusing and green plant regeneration from anthers of indica rice cultivars (Oryza sativa L.) Rasi, Tellahamsa (TH), Ptb-33 and Getu was standardised. The N6 medium supplemented with 5% maltose, 10.0 mg/l AgNo3, 2.0 mg/l 2,4-D and 0.5 mg/l Kn gave high frequency callusing (14-34%) within 20 days compared to control (2%) on N6 medium. Anther derived callus when transferred to MS regeneration medium with 1.0 mg/l IAA, 2.0 mg/l BAP, 0.5 mg/l Kn and 3% sucrose produced only albino plants except Ptb-33. The calli when subcultured on N6 medium with 1.0 gm/l proline and 2.0 mg/l 2,4-D (N6P) for four weeks, transferred to MS regeneration medium gave upto 50% green plantlets in Rasi and 60% in Ptb-33. The anther callus induced on N6P medium and transferred to MS regeneration medium exhibited 50% and 85% green plantlets in Rasi and TH, respectively. There was significant increase in the frequency of green plant regeneration (85%) in TH on N6P induced calli compared to control (10%), media devoid of proline. These preliminary studies suggest that proline may play a significant role in enhancing green plant regeneration from anther derived calli of indica rice. Which can be exploited in anther culture breeding programms.

P-1065

Effect of Thidiazuron on Regeneration from 'Half-Seed Explants' of *Capsicum annuum* L. M.L. Binzel[1], N. Sankhla[1,3], D. Sankhla[2], T.D. DAVIS[2] & S. Joshi[3]. Texas Agric. Expt. Sta., 1380 A&M Circle, El Paso, TX 79927[1], Texas Agric. Expt. Sta., 17360 Coit Rd., Dallas, TX 75252-6599[2], J.N. Vyas Univ., Jodhpur 342001 India.

Gene transfer technology offers potential for the development of transgenic peppers containing genes which confer various resistances. Pronounced intercultivar differences in regeneration potential coupled to the lack of efficient *in vitro* regeneration protocols have limited the application of biotechnology to pepper. This report describes work aimed at developing an efficient regeneration protocol using 'half-seed explants' of a mild long-green chile (cv. New Mexico 6; NM-6) and a pungent chile (cv. Rajpur-Hirapur; RH). Seeds were soaked in H_2O or 10 µM thidiazuron (TDZ) for 1-6 d. The seeds were cut into two pieces so that one portion contained the proximal end of the hypocotyl and the radicle. This 'half-seed explant' was cultured on MS basal medium or MS medium with TDZ at 0.01-1 µM. In the controls, 1-2 leafy buds formed at the cut surface of the hypocotyl in 2-5% of the explants within 15-20 d. TDZ increased the percentage of explants forming buds (60-90%) and the number of buds (7-14) per explant. A 72 h soak in TDZ was optimal for regeneration. The continuous presence of TDZ in the medium, however, inhibited elongation of the rosette-like leafy buds. Generally, NM-6 was more responsive to TDZ than RH. Within 3-5 weeks after transfer to magenta boxes containing vermiculite and soil (1:3) moistened with MS medium, 70-85% of the rooted hypocotyls with leafy buds developed 1 or 2 elongated shoots. After transfer to pots, the plantlets grew into normal pepper plants.

P-1066

LEAVES ROOTS AND SUSPENSION CULTURED CELLS OF RICE (*ORYZA SATIVA* L.) AND ITS GENOMIC POLYMORPHISM ANALYSED USING RAPD

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We have investigated the occurrence of genomic polymorphisms in the DNA from the following rice (*Oryza sativa* L.) material: (I) leaves and roots from seed-derived plant, (II) seed-derived calluses and embryogenic cell suspension cultures. Eight cultivars of the Idica and Japonica type were used. In all cases the DNA was derived from a single aseptically grown seed and at least six repetitions were made. PCR amplification with different random primers produced RAPDs (Random Amplified Polymorphic DNA) that was analysed by agarose gel electrophoresis. Principal coordinated analysis of the polymorphic DNA bands showed that: (a) all tested rice cultivars have an homogeneous genetic constitution; (b) in all cases, DNA from leaves, roots, calluses and embryogenic cells from the same plant are significantly different from each other; (c) the genomic constitution of dedifferentiated calluses is similar to that of roots; (d) DNAs from embryogenic cell suspension cultures are significantly different from all other analysed samples.
The expected genomic difference due to the known genetic distance among the analysed cultivars are also verified. The results suggest that the rice genome undergones changes during plant differentiation or in vitro cell culture.

P-1067

Multiple Virus Eradication from Potato. C. ZAPATA, J. C. Miller and R. H. Smith. Dept of Soil & Crop Sci (C.Z., R.S.) and Hort Sci (J.M.). Texas A&M Univ, College Station, TX 77843

A tissue culture method was developed for the eradication of 3 of the most important potato viruses from the Russet Norkotah variety and 2 strains (TXNS 278 and TXNS 112). The method combined the use of liquid medium, thermotherapy and chemotherapy. Initially, virus-free plants were inoculated with PVX, PVY, and PVS and, after 10 d tested by ELISA, to establish initial virus concentrations. Stem sections of inoculated plants were planted in liquid medium containing MS inorganic salts, vitamins, and ribavirin (40µM or 80µM) at room temperature for 5 d. Half of the plants were then subjected to thermotherapy. After 6 weeks, the uppermost node was removed and transplanted onto a solid medium. Plants were tested initially and 6 weeks later using ELISA to identify the virus-free plants.
Ribavirin alone eradicated viruses from some plants; however, more virus-free plants were obtained using thermotherapy. The use of liquid medium enhanced plant growth and allowed production of more than 5 plants per treated stem. This procedure is less time-consuming, and requires little expertise, compared to the meristem culture method to obtain virus-free plants. The method resulted in production of more than 10% virus-free plants.

P-1068

ANTHER CULTURE STUDIES FROM SALT TOLERANT CULTIVARS OF INDICA RICE, S.K.ANITHA AND G.M.REDDY, CENTRE FOR PLANT MOLECULAR BIOLOGY, OSMANIA UNIVERSITY, HYDERABAD, A.P., INDIA.

Callus was initiated from anthers of Korgut Pokkali salt tolerant cultivars Azgo moderately tolerant cultivar and Tellahamsa a susceptible cultivar on N6 medium with 5% Maltose, 2mg/1 2,4-D and 0.5mg/1 Kn. High frequency of callusing was observed in Korgut (16%) followed by Pokkali (5%) Azgo (0.5%) and Tellahamsa (5%) N6 medium supplimented with maltose was found to be superior for callus initiation over the sucrose. Successful plant regeneration was obtained from Korgut (45%), Pokkali (30%) and Tellahamsa (30%) on MS medium with 1IAA 2BAP, 0.6 Kn. Azgo did not exhibit any regeneration. Calli were subjected to 50, 100,150 & 200mM Nacl for four weeks and sub sequently regeneratated on MS medium. Succe ssful regeneration was obtained from Korgut and Pokkali upto 100mM Nacl stress whereas salt susceptible Tellahamsa failed to regenerate. But Korgut exhibited high frequency of regeneration (30%) compared to Pokkali (8%). These studies suggest that Korgut a slat tolerant cultivar exhibited efficient plant regeneration with and without salt stress compared to Pokkali.Further studies are in progress with regard to the evaluation of the progeny.

P-1069

Genetic Analysis of Salinity Tolerance in Rice

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Salt tolerant cultivars Korgut and Pokkali, moderately tolerant cultivar Azgo and, susceptible cultivars Shito and Tellahamsa were crossed in all posible combinations. Parents and F1s were screened in normal and saline nutrient solution and genetic parameters were compared. Additive and dominent gene effects were significant for all the characters except grain yield for plant. Yield related character showed overdominance in saline conditions. General and specific combining abilities showed that Korgut and Pokkaliwere best combiners. Characters like plant height, tiller number, percentage of seed set, percentage of productive tiller, panicle length, grain yield per plant showed high heritability values under salinity. These traits are being used for selection of salt tolerant cultivars. Present studies suggest that the crosses between Tellahamsa x Pokkali, Tellahamsa x Korgut exhibited better tolerance compared to their parents. Segregating generations of these crosses at F3 , F4 were evaluated hydroponically with two week old seedlings on salinized culture solution (16 Nacl : 1 Cacl2 , EC 12 ds/m) for 3 weeks in the green house, salt tolerant progeny were selected, and transfered to pots for further studies. This method allows to select more tolerant recombinent lines compared to either parents.

P-1070

Salt-Responses in Oryza sativa seedlings : Role of Calcium and Gibberelic acid in Salt Toxicity.

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Salt stress imposed by NaCl, Na_2SO_4 (200 mM), KCl and K_2SO_4 (150 mM) inhibited the seed germination, root and shoot lengths considerably in salt sensitive and tolerant cultivars of rice seedlings. In presence of low concentrations of Ca^{2+} (1 mM) and GA_3 (1 mg/l), the frequency of germination was reduced by 54-75%, shoot length by 32-91% and root length by 67-96%. But, on increasing the Ca^{2+} to 9 mM and GA_3 to 10 mg/l at same salt concentrations restored the frequency of germination, improved plumule emergence, root and shoot lengths significantly both in salt sensitive and tolerant cultivars. Accumulation of proline was only 1 to 3.4-folds under salt stress in the seedlings. Both Ca^{2+} (9 mM) and GA_3 (10 mg/l) increased the accumulation of proline by 1 to 1.8-folds in the presence of salt stress but not in the absence. But ABA alone could induce proline in rice in the absence of salt stress, suggesting that ABA accumulation may be necessary for salt induced proline elevation. The activities of γ-glutamyl kinase, the rate limiting enzyme and Δ'-pyroline-5-carboxylic acid reductase, involved in final step of proline biosynthetic pathway, were detected in the extracts of seedlings after ammonium sulphate precipitation. The activities of these two enzymes increased to 2 to 2.5-folds with NaCl treatment indicating that the proline pathway is stimulated by salts. Amileoration of salt stress by supplemental Ca^{2+} and GA_3 may be exerted through preventing Na^+ related changes in Ca^{2+} homeostasis.

P-1071

The Influence of Osmoticum on Protoplast Yields of Selected *Eucalyptus dunnii* Maid. Clones.

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Eucalyptus dunnii Maid. is an important species for biomass and pulp production possessing both rapid growth habit and frost tolerance. However, factors such as poor seed production and restricted germplasm availability limit use of this species. Development of a viable protoplast culture system provides a means for increasing available genetic variability. The youngest developing leaves from *in vitro* cultures of selected clones were sectioned into 1mm segments while immersed in a polyphenol absorbing solution, followed by treatment for 1 hr in protoplast washing solution (PWS) containing 0.6M, 0.8M, or 1.0M sorbitol. Tissue was incubated for 3 hr in cellulase/macerase solution containing 0.6M, 0.8M, or 1.0M sorbitol. Digests were filtered, washed in PWS with respective sorbitol concentrations, centrifuged, and pellets were resuspended in PWS. Protoplasts were collected by centrifugation in 20% sucrose solution, stained with Evans blue, and yields determined. Overall, the number of viable protoplasts decreased with increasing osmoticum. However, yields varied with specific clone, and viability was more affected than protoplast number. The yields obtained in this study are sufficient for successful development of protoplast culture for this species.

P-1072

Expression of Anthocyanins in Bilberry and Huckleberry Callus Cultures.

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The intensely colored edible fruits of genus *Vaccinium* are valuable resources for anthocyanins, potential natural food colorants. In vitro, cell cultures derived from vegetative parts from *Vaccinium* species in general express a simpler anthocyanin profile as compared to the fruit. The regulatory mechanisms determining the highly organ specific expression of anthocyanins in vivo seem to be operative in cell cultures also. Bilberry (*V. myrtillus*) has a complex anthocyanin profile. A reverse phase HPLC analysis of anthocyanins revealed fifteen peaks in bilberry fruit extract. Bilberry callus derived from vegetative parts had a simpler anthocyanin profile and contained three major peaks. A yellow fruited selection from huckleberry (*V. pahalae*) does not accumulate anthocyanins in the fruits. However, juvenile stems and leaves are intensely colored and contain two major anthocyanins. The anthocyanins in callus cultures derived from leaves were comparable to the vegetative explants. The characterization of anthocyanins expressed in vitro in bilberry and huckleberry cultures is presented.

P-1073

Anthocyanins in *Ocimum basilicum* cv. Purpurascens In Vitro.
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Ocimum basilicum cv. Purpurascens (Sweet basil 'Purple Ruffles') family *Labiatae* is a pleasantly fragrant, edible herb with deep purple, glossy foliage. The intensely colored plant could be a potential resource for anthocyanins in vivo and in vitro. The anthocyanins in vivo have a relatively high stability compared to cranberry anthocyanins. We have established callus cultures from sweet basil 'Purple Ruffles' which accumulate anthocyanins in the dark. Growth regulators were found to have a distinct effect on callus induction and pigmentation. A reverse phase HPLC analysis of anthocyanins revealed six major peaks in the leaf extract. The profile of anthocyanins from callus cultures was comparable to in vivo extract. Analysis of the acid hydrolysate of the total extract indicated that the major aglycone was cyanidin.

P-1074

Physical Microenvironmental Effects on Anthocyanin Production in Cell Cultures of *Ajuga pyramidalis* 'Metallica Crispa'.
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The physical microenvironment of a cell culture, including light and temperature, can be deliberately manipulated to affect growth and anthocyanin accumulation in vitro. The productivity of the natural pigment culture system has a direct bearing on the feasibility of its scale-up for industrial use. The effects of light intensity, source *(wavelength)*, and photoperiod and temperature on *Ajuga pyramidalis* 'Metallica Crispa' cell and suspension culture growth and anthocyanin production were evaluated in this study. No significant effects on callus induction and growth from *Ajuga* leaf explants grown under cool white were detected under continuous light, alternating light/dark *(16/8 hrs)*, or continuous dark photoperiods. Anthocyanins were expressed only in calli in treatments receiving light. Callus grown under higher photosynthetic photon fluxes *(PPF) (up to 150 $\mu M\ m^{-2}\ s^{-1}$)* tended to accumulate greater concentrations of anthocyanin. Cool white, blue *(peak λ=480 nm)*, and red *(660 nm)* fluorescent lamps supported cell culture growth better than did ultraviolet *(313 nm)*. Blue stimulated anthocyanin accumulation better than cool white, ultraviolet, and red light respectively. Cultures incubated at 25°C exhibited faster cell growth and anthocyanin accumulation than at 15°C.

P-1075

Triterpenoid Composition of In Vitro Tissues of Maritime Pine (Pinus pinaster Ait).
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Lipidic compounds, namely phytosterols, are important plant membrane constituents. Quantitative and qualitative alterations on phytosterol composition have been related with some physiological and functional alterations on plants, like flowering. On the other hand phytosterols, namely sitosterol, are an important source of raw material for the production of therapeutical steroids. Therefore the knowledge of the phytosterol composition is important to a better understanding of some plant functional aspects and to manipulate sterol production via *in vitro* controlled, cultured conditions.
The triterpenoid composition of maritime pine calli was studied. Calli were established from hypocotyls of aseptically grown seedlings, and maintained in MS medium containing 2% (w/v) sucrose, NAA (5 $mg.ml^{-1}$), kinetin (0.5 $mg.ml^{-1}$), ascorbic acid and citric acid (100 $mg.ml^{-1}$, each), pH 5.7, in a day/light photoperiod of 16/8 h. Calli 5 months age actively grown were analyzed and compared with calli that, althoug with the same age, had lost the growth competence and initiated a senescence period over 4-5 months. Campesterol, sitostanol and sitosterol (more than 75% of the sterol fraction) are the major sterols.

P-1076

Synthesis and Accumulation of Essential Oils in *In Vitro* Regenerated Shoots and *Calli* of *Chamaemelum nobile* L.
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Calli of *Chamaemelum nobile* L. were induced from leaf segments cultured on MS or B5 basal media supplemented with 2,4-D or NAA and one of the citokinins: KIN, BA or ZEA. Regeneration of shoots and roots was obtained from calli subcultured on MS medium supplemented with NAA and BA. GC-MS analysis of the essential oil obtained by hydrodistillation of regenerated shoots maintained *in vitro* for 28 weeks revealed synthesis and accumulation of terpenic compounds namely: octen-3-ol, dimethylbenzene, 1,8-cineole, camphor, azulene, caryophyllene, germacrene D, farnesene, geranyl propionate and benzophenone. A very different and poor composition was recorded for hydrodistillate obtained from *calli*. Benzene acetaldehyde, octen-3-ol, ethyl benzene, dimethyl benzene and camphor were the main compounds identified by GC-MS.

P-1077

Enhancing Production of Artemisinin in Transformed Roots of *Artemisia annua*. P. WEATHERS, T. Smith, D. Hemmanvanh, E. Follansbee, J. Ryan, R. Cheetham. Biology & Biotechnology Dept., Worcester Polytechnic Institute, Worcester, MA 01609.

The potent antimalarial, artemisinin (AN), is produced in transformed roots of the Chinese herb, *Artemisia annua*. A variety of factors including nitrate, phosphate, GA_3, temperature, light, and carbon have been evaluated for maximum biomass productivity. The elicitor chitosan is effective in stimulating synthesis of high levels of artemisinic compounds. Synergistic effects of these factors for optimizing production of AN are under investigation. The kinetics of AN production, relevant to biomass and other artemisinic compounds, also show that an unusually early (3-6 days) peak of AN synthesis in clone YUT16 just prior to exponential growth. If the factors controlling this high level of productivity can be identified and then applied to densely packed root cultures, the cost of production will be significantly lowered making AN economically attractive as a therapeutic.

P-1078

Regeneration and Transformation in Sunflower (*Helianthus annuus* L.) Mature Cotyledons. C.M. BAKER and C.D. Carter. Plant Science Department, Northern Plains Biostress Laboratory, Box 2140-C, South Dakota State University, Brookings, SD 57007

Without an efficient regeneration method, application of transformation techniques for the improvement of sunflower (*Helianthus annuus* L.) are limited. The method must be reproducible with high efficiency, and produce large numbers of shoots which are well developed and exhibit few signs of hyperhydricity. Plants must be obtained. A two step regeneration system has been developed using mature cotyledons from one day old seedlings. First, cotyledons are placed on medium with 0.5 mg/l NAA and 1 mg/l BA for 4 days to induce shoots. Second, cotyledons are transferred to medium with 0.01 mg/l NAA, 0.5 mg/l BA, 0.1 mg/l GA_3, and 0.43 mg/l $AgNO_3$ to allow shoots to develop. Shoots are rooted. Factors affecting this shoot regeneration and rooting are plant growth regulator concentrations and types, shoot development, and carbohydrate concentration. Transformation studies using the particle inflow gun are being conducted. Factors affecting the transformation efficiency are explant age, preparation for bombarding, helium pressure, number of times each plate is shot and selection method. Transient GUS expression has been obtained.

P-1079

Effect of Donor Plant and Culture Factors on Transient Gene Expression in Alfalfa Following Microprojectile Bombardment. L.-N. TIAN[1,2], D.C.W. Brown[2], and J. Webb[1]. [1].Department of Biology, Carleton University, Ottawa, Ontario Canada K1S 5B6. [2] Plant Research Centre, Central Experimental Farm, Ottawa, Ontario Canada K1A 0C6

Explants of alfalfa, including petioles, stems, roots, leaves, embryogenic callus, and somatic embryos were used to investigate the potential for transformation using these explants as *in vitro* bombardment targets. GUS gene expression was quantifiable in these explants, but the stage of the explants in culture strongly influenced GUS expression. Bombardment of petioles after day 5 in culture gave the highest GUS gene expression of the 0-20 day period tested. Bombardment of petioles in two different types of media, which induce dramatically different number of somatic embryos, resulted in substantially different levels of GUS gene expression. The results were interpreted to mean that induction and (or) development of a cell's competence for somatic embryogenesis does not necessarily correlate with the cell competence as a bombardment target. Bombardment of proembryogenic tissue on embryo development medium resulted in fewer cells expressing the GUS gene than in mature somatic embryos. The transient expression of the GUS gene in petioles decreased quickly in the 15 days following bombardment, indicating poor survival of these cells or low transition frequency from transient expression to stable expression. Bombardment of petioles of ten alfalfa genotypes, including *Agrobacterium*-transformable and recalcitrant alfalfa genotypes, as well as diploid and tetraploid genotypes, gave a similar level of GUS expression. These results suggest that genotype is not a critical factor with respect to transient gene expression after particle bombardment delivery of DNA.

P-1080

Plant Regeneration and *Agrobacterium*-mediated Transfer of *RolC* Gene in *Salpiglossis sinuata* L. L. Wang and C.W. LEE. Department of Plant Sciences, North Dakota State University, Fargo, ND 58105.

Plant regeneration and genetic transformation for *rolC* gene in *Salpiglossis sinuata* L. were investigated. Shoot organogenesis from callus tissues was most efficient when they were cultured on Murashige and Skoog (MS) medium supplemented with 0.5 μM naphthaleneacetic acid (NAA) and 2.0 μM kinetin. Root initiation on *in vitro* grown shoots was best accomplished by using 0.5 μM NAA only in the MS medium. Leaf discs from five different salpiglossis genotypes were infected with *Agrobacterium tumefaciens* strains LBA4404 and EHA101 carrying *rolC*, *GUS*, and *NPTII* genes. Both *Agrobacterium* strains and salpiglossis genotypes affected plant transformation efficiency. Of the two *Agrobacterium* strains tested, EHA101 provided a higher transformation frequency than LBA4404. Among the five different salpiglossis genotypes used, the chasmogamous line with solid yellow flowers (*ccrrD*-) showed the highest transformation efficiency. The presence of the *rolC* gene in transgenic plants was verified by histochemical *GUS* assay, Southern blotting, and PCR analysis. Some transgenic plants with *rolC* gene flowered *in vitro*.

P-1081

Protoplast-mediated Transformation of Peanut (*Arachis hypogaea*) for Virus Resistance. Z. Li[1], A. Xing[1], M. CHENG[1], R.L. Jarret[2] and J.W. Demski[1]*. [1]Dept. of Plant Pathology, Georgia Station, Univ. of Georgia; [2]USDA-ARS Plant Genetic Resources Conservation Unit, Georgia Station, Griffin, GA 30223, USA

Efficient plant regeneration from peanut (*Arachis hypogaea*) protoplasts has been achieved. Co-cultivation of cotyledonary protoplasts from 10 US cultivars and breeding lines with peanut nurse cells resulted in the formation of callus colonies. After transfer to a regeneration medium, up to 80% of protoplast-derived colonies formed multiple shoots within a period of three months. Protoplast-derived plants, grown to maturity in the greenhouse, were fertile. An efficient protocol for protoplast-mediated transformation of peanut has been developed. Conditions for efficient gene transfer into peanut protoplasts using electroporation have been defined utilizing a poration medium containing a glycine buffer. Transient GUS expression was increased 8- to 430-fold using the newly-devised poration medium, when compared to other commonly-used poration media. A large number of transformed colonies containing the reporter GUS gene and selectable marker genes (*nptII*, *hph* and *bar*) have been obtained. Southern blot hybridization and PCR analyses confirmed the integration of intact transgenes into the peanut genome. Transgenic plantlets have been recovered and are being rooted for further evaluation. By using this efficient transformation system, a number of virus coat protein genes have been introduced into major US peanut cultivars for the induction of virus resistance.

P-1082

Transformation of Elite Maize Inbreds by Microprojectile Bombardment of Type I Callus. JOHN DAWSON, Erik Dunder, Narendra Palekar and Jan Suttie. CIBA Biotechnology, POB 12257, Research Triangle Park, NC 27709

An efficient transformation system was developed for elite maize inbreds using microprojectile bombardment of type I callus. Use of the type I callus allows for transformation of a readily available, uniform target. The system was optimized for stable transformation using expression of the anthocyanin regulatory genes, C1 and B-peru, to generate red, multi-celled sectors. A selectable marker gene, bar, driven by the CaMV 35S was co-transformed. Various transformation parameters which tested gene delivery (particle velocity) and tissue treatment (osmoticum, auxin levels, wounding) were assessed. The variables were optimized using an easily cultured CIBA elite inbred. Inbreds were transformed with a synthetic gene encoding an insecticidal crystal protein from Bacillus thuringiensis. Maize T0 plants and T1 progeny were generated and showed high insecticidal activity against European corn borer.

P-1083

High Frequency Co-Transformation of Embryogenic Peanut Cultures. H.D.Wilde, Z.V. Magbanua, and W.A. PARROTT. Department of Crop and Soil Sciences, University of Georgia, Athens, GA 30602.

Methods for transformation of a commercial peanut cultivar (VC1) were optimized using the ß-glucuronidase and hygromycin phosphotransferase genes of plasmid pTRA140. The target tissue was an embryogenic suspension culture, which produced somatic embryos that converted into plants at a frequency of 20.2%. Peanut cultures were transformed biolistically with pTRA140 and regenerated into plantlets within 6 months. Co-transformation with a second plasmid was explored as a means of introducing genes of interest into peanut. A plasmid containing the nucleocapsid (N) gene from a tomato spotted wilt virus (TSWV) peanut isolate was combined with pTRA140 (1:1 molar ratio) and cultured tissue was bombarded with this mixture. Clonal cell lines were established from individual cell clusters which proliferated in the presence of 20 mg/l hygromycin. Over 500 independent transformants were recovered from 23 bombarded plates. Of 141 cell lines analyzed by PCR to date, 83% contained the N gene. Higher ratios of the second plasmid (1:3 and 1:6) gave 100% co-transformation. Southern blot analysis showed that the number of copies of the N gene integrated into the genomes of transgenic cell lines ranged from 2 to approximately 20 . The germination of co-transformed somatic embryos is in progress.

P-1084

In vitro Regeneration and Potential Transformation of Peanut (*Arachis hypogaea* L. cv. Okrun). **J. PONSAMUEL**, D.V. Huhman, B.G. Cassidy, R.S. Nelson, and D. Post-Beittenmiller. Plant Biology Division, The Noble Foundation, P.O. Box 2180, Ardmore, OK 73402, USA.

A protocol for rapid and efficient shoot regeneration from immature embryonal axes of *Arachis hypogaea* L. cv. Okrun was developed in order to establish an *Agrobacterium*-mediated transformation system. Plumular crown explants sliced at the cotyledonary node and containing a small fragment of cotyledon were isolated from the seed's 5th developmental stage. Initially these explants were cultured on B5h medium containing 1mg/l 2,4-D. After 5 d of incubation the explants were trimmed to remove the pre-existing plumular shoot derivatives before subculturing onto MS-based medium containing 30 µM BAP and 5 µM NOA as hormonal supplements. A mantle of meristemoids developed around the explants within 18 d after subculture. About 16 meristemoids differentiated per 1 cm^2 and 8% of these meristemoids developed into shoots. The rest appeared "dormant." After the first set of shoots were harvested, the "dormant" meristemoids were induced to differentiate into shoots when subcultured onto MS-based medium with 1 µM brassin and 1µM indoleacetonitrile. After 50 d of subculture, the shoots at the two leaf stage were isolated and rooted in 1/2X MS-based medium with 10 µM NOA. A 16 hr photoperiod of 54 µm photons m^{-2} s^{-1} from cool white fluorescent lights and a temperature regime of 26±2°C was provided for all the culture stages. The plumular explants were treated with EHA105 containing *bar* and *npt* genes and the shoots were regenerated using the above protocol with selection against *Agrobacterium*. The shoots were rooted without selection for transformants and transferred to a greenhouse for acclimation and establishment. Chlorophenol red screening for bialaphos resistance was positive for some of the plants from *Agrobacterium*-treated tissue and negative for all the non-transgenic controls. Molecular analyses are now in progress.

P-1085

Genetic Transformation of Seashore Mallow by Agrobacterium tumefaciens. J.D. RAO, D.M. Seliskar, and J.L. Gallagher. Halophyte Biotechnology Center, College of Marine Studies, University of Delaware, Lewes, DE 19958.

Seashore mallow, Kosteletzkya virginica (Malvaceae), is a C3 halophyte which inhabits brackish marshes along the mid to southern Atlantic coast of the U.S. Genetic transformation of this species was accomplished by co-cultivation (48-72 hrs) of leaf, shoot apices, stem, and hypocotyl explants from 8-day-old seedlings with Agrobacterium tumefaciens LBA 4404. This is a disarmed strain harboring a binary vector pBI 121 carrying the CaMV 35S promotor-β-glucuronidase (GUS) gene as a reporter gene and NOS promotor-neomycin phosphotransferase NPTII gene as a selection marker. Following co-cultivation, explants were transferred onto MS media supplemented with carbenicillin and kanamycin. Shoot buds/shoots were developed from hypocotyl explants in the presence of thidiazuron (TDZ), carbenicillin (300 mg/l), and kanamycin (50 mg/l) after six weeks of culture. Friable yellow callus was obtained from leaf discs cultured on MS + BAP (2.0 mg/l) + IAA (0.1 mg/l). Different parameters, such as co-cultivation conditions, kanamycin sensitivity, pre-culture of explants, and shoot regeneration from hypocotyl explants, were studied. A greater number of blue spots, indicating GUS expression, appeared in the precultured explants than in the control explants. Histochemical assays revealed that the GUS gene is expressed in all explants tested after four weeks of culture. Further studies are in progress. The protocol may be useful for improving seashore mallow through genetic manipulations.

P-1086

Transient Expression of CAT and GUS Activities in Maize Embryos and in Germinating Pollen. [2]N. TSENGWA and [1]J.A. Saunders and [1]R. Patel and [2]M.S. McIntosh. [1]USDA/ARS/CSL Bldg 9, Rm 5, Beltsville, MD 20750. [2]University of Maryland, Agronomy Dept. College Park, MD 20742

Various genes of interest have been cloned and isolated for the purpose of crop improvement, however, the techniques to transfer them into many important agronomic crops, i.e. monocots, are still limiting. In this study, two procedures were used to electroporate chloramphenicol acetyl transferase (CAT) and β-glucuronidase (GUS) genes into maize tissue. Electroporation of immature pretreated zygotic embryos using an exponential wave pulse with the pBI221 GUS plasmid resulted in transient expression of GUS activity in the embryo tissue. Permeabilization of germinating pollen by electroporation was also accomplished with a square wave pulse in the presence of pCaMV CAT and pAT13 GUS plasmids which resulted in pollen expressing both activities. The expression of these reporter genes in pollen demonstrates that foreign DNA can be introduced into the germinating maize pollen, thus allowing the opportunity for direct production of transformed seeds by pollination. The use of pollen as a transformation system of this type has been demonstrated in tobacco and by-passes the problems associated with prolonged protoplast regeneration and tissue culture periods. On the other hand, the introduction of DNA directly into the maize embryos offers an alternate route whereby transformed embryos can be grown directly into plants. We are currently pollinating the flowers using transformed pollen and growing putatively transformed embryos into full plants to screen resulting tissue for transformation.

P-1087

Optimization Of Particle Bombardment Conditions For Long Term Stable Expression Using GUS Gene In Wheat. WEN CHUNG WANG and David Marshall. Texas A.&M. University, Research and Extension Center at Dallas, Texas 75252-6599.

Our experiments were directed toward increasing the frequency of stable transformation in hard red winter wheat using particle bombardment procedure. We have evaluated the different bombardment conditions (distance, pressure and gold particle size), cultures (shoot competent cell and immature embryo, 14-17 DAP) and two GUS gene constructs (pNG1 and pAHC25) after 2 days and 30 days of bombardment using GUS gene expression. For pAHC25, the optimal conditions for shoot competent cell by counting the blue spots 2 and 30 days after the bombardment gave average 452 and 7 spots per plate, respectively. For immature embryo, the optimal condition after 2 and 30 days of bombardment gave average 103 and 2.1 spots per embryo, respectively. Though, the optimal condition gave the highest number of blue spots after 2 days of bombardment also gave the highest number of blue spots after 30 days of bombardment for both shoot competent cells and immature embryo, but the numbers of blue spots after 30 days of bombardment were very low. The optimal system for stable integration of the gene can be and should be evaluated after the gene has been transferred into the cells and maintained stably for long term. Efforts are now underway to recover stable transformants in hard red winter wheat. The comparisons of parameters of bombardment, two gene constructs, and cultures after 2 days and 30 days of bombardment will be discussed.

P-1088

Effect of Timentin for Controlling Agrobacterium tumefaciens following Cocultivation on Select Plant Species. T.W. ZIMMERMAN. University of the Virgin Islands, Agricultural Experiment Station, RR 2 Box 10,000, Kingshill, St. Croix, USVI 00850.

Timentin, a proprietary product of SmithKline Beecham Pharmaceuticals, is a mixture of ticarcillin and clavulanic acid and was used in a ratio of 50:1 (w/w), respectively. Filter sterilized Timentin was used at 0, 50, 100, 200 and 400 mg/liter in culture media to study its effect on micropropagation, organogenesis, somatic embryogenesis and counterselection of Agrobacterium tumefaciens strains. Petunias and hibiscus, micropropagated with up to 400 mg/liter timentin, produced no significant reduction in propagules after four monthly passages on medium containing Timentin. Organogenesis from petunia leaf disks and somatic embryogenesis from papaya hypocotyls were likewise not significantly influenced by the addition of up to 400 mg/liter Timentin. The A. tumefaciens strains LBA4404, C58, EHA101 and EHA105 had reduced colony development with 50 mg/liter timentin and control of the bacteria at 100-400 mg/liter Timentin. Following cocultivation with petunia leaf disks for two days, counterselection of A. tumefaciens strains was effective at 100-400 mg/liter Timentin. Timentin is effective at 100 mg/liter as an alternative to carbenicillin in controlling A. tumefaciens strains following cocultivation.

P-1089

Rice transformation using *Agrobacterium* and the shoot apex. S.H. PARK and Roberta H. Smith. Dept of Soil & Crop Sci Texas A&M Univ, College Station, TX 77843

Work on rice transformation has focused on the Maybelle cultivar using *Agrobacterium tumefaciens* containing plasmids for the 35S promoter on the *bar* gene and the actin promoter on the *bar* gene. Experiments to determine the survival rates or kill curves of isolated shoot tips on media containing the herbicide glufosinate-ammonium (ppt) at 0, 0.05, 0.1, 0.5, and 1.0 mg/l have established that no shoot tips survived on 0.5 or 1.0 mg/l ppt. Survival rates were 45, 66, 66, 0, and 0%, respectively. However, after shoot tips had been cocultivated with *A. tumefaciens* containing the gene for resistance to ppt, survival rates on 0.5 mg/l ppt were 1.8, 2.8, 2.9 and 7% in 4 different replications; overall 20 out of 724 (2.8% shoots) survived the ppt selection.
PCR analysis of 7 primary rice plants and Southern blot analysis of DNA (BamHI digest) from 10 primary rice plants indicates that the *bar* gene is present in at least 5 plants. Southern blot analyses (XbaI digest) and leaf application of Ignite on some progeny from two of these plants established that the bar gene and NOS/NPTII gene were present and the plants resistant to Ignite in a 4:1 and 2:1 Mendelian pattern.

P-1090

Insect Chitinase-mediated Resistance to Tobacco Budworm (*Heliothis virescens*) in Transgenic Tobacco Plants. X. DING[1], L. Johnson[1], F. White[1], B. Gopalakrishnan[2], K. Kramer[3], and S. Muthukrishnan[2]. Depts. Plant Pathology[1] and Biochemistry[2], Kansas State Univ., Manhattan, KS 66506 and [3]U.S. Grain Marketing Res. Lab., A.R.S., U.S.D.A., Manhattan, KS 66502.

Although insect control on plants appears possible through the use of transgene technology, one limitation is a shortage of useful genes for this purpose. A possible target is the insect gut, which contains chitin, and where chitinases are temporally expressed during molting. Constitutive expression of the appropriate chitinase in a transformed plant thus might have negative effects on development of larvae feeding on it. We tested this using a chimeric construct encoding a chitinase cDNA cloned from *Manduca sexta* that was introduced via *Agrobacterium tumefaciens*-mediated transformation into tobacco. High-expressing transgenic plants were self-fertilized, and the progeny were examined both for effects on the growth of *Heliothis virescens* larvae and on feeding damage. Transgenic plants constitutively expressing the chitinase exhibited enhanced resistance to insect feeding. Larval growth rate and survival were significantly reduced. The use of this insect-derived chitinase gene represents a promising alternative to the use of other transgenes for insect control.

P-1091

Transformation of *Sorghum bicolor* L. TAE-SEOK KO and Roberta H. Smith. Dept of Soil & Crop Sci Texas A&M Univ, College Station, TX 77843

Shoot apices isolated from sorghum seedlings, CVS cultivars, RTx430 and BTx319,7 were co-cultivated with *A. tumefaciens* EHA101 carrying gus, nosnptII and bar genes under the control of CaMV 35S or rice actin 1 promoters. Inoculated shoots with CaMV 35S-gus were recovered and rooted plants (14%) were obtained in 3-5 weeks. GUS activity in regenerated plants was undetectable in histochemical assay. The frequency of transformation, estimated from PCR amplification of introduced genes was 20%. RI plants derived from a putative transformant indicated the presence of both foreign genes. Genomic restriction and Southern analyses confirmed that the introduced genes were stably incorporated into high molecular weight plant genomic DNA and transmitted into R1 and R2 generations. Experiments were done with two plasmid constructs containing either a 35S or *Act 1* promoters/bar gene conferring resistance to phosphinothricin. Transformed plants were selected on 0.5 mg/l PPT. Data from genomic Southern analyses and enzyme assays for Ro plants proved that the introduced gene was stably integrated into plant genomic DNA and expressed. Analysis of R1 plants to confirm the sexual transmission of the introduced herbicide gene and stable expression in progeny is in progress.

P-1092

Transformation of *Solanum brevidens* Using *Agrobacterium tumefaciens*. T.-H. ANNIE LIU, Loren C. Stephens and David J. Hannapel. Department of Horticulture, Iowa State University, Ames, IA 50011-1100.

Leaf pieces of *in-vitro* cultured plantlets of the wild potato species *Solanum brevidens* Phil. were cocultivated with *Agrobacterium tumefaciens* that contained *nptII* and GUS genes on the disarmed plasmid pBI121. Independent transgenic shoots were regenerated from both solidified and liquid medium that contained 50 $mg \cdot l^{-1}$ kanamycin. Two *Agrobacterium* strains were investigated for transformation efficiency. GV2260, which contained p35SGUSINT, resulted in a 11% transformation frequency, compared with 1% using LBA4404. Transformation rate were 7% in liquid culture and 3% on solidified medium. All kanamycin-resistant, putatively transformed plantlets were confirmed positive by histochemical GUS assays. GUS activity in 22 independently transformed plants was quantified by fluorometric assay. Southern analysis of randomly selected transgenic plants showed that each transgenic plant contained at least one copy of the GUS gene.

P-1093

Transformation of *Euphorbia lathyris* by *Agrobacterium rhizogenes*. E. Follansbee, R. CHEETHAM, and P. Weathers. Department of Biology and Biotechnology. Worcester Polytechnic Institute. Worcester, MA 01609

Euphorbia lathyris (Gopher Purge) produces two potential anti-tumor agents and possibly a rodent repellent. Infection of *Euphorbia lathyris* seedlings by *Agrobacterium rhizogenes* ATCC 15834 has yielded a number of hairy root clones. The growth and product production characteristics of these transformed root clones have been studied and compared to normal plants. The growth rates are significantly higher than normal roots. Cultures on plates and in different liquid media have been compared to growth in a nutrient mist bioreactor. Regeneration of whole plants from these roots has been attempted: normal *E. lathyris* regenerates easily, but the altered endogenous auxin levels, and phytohormone sensitivity of the transformed tissues must be counteracted with cytokinins or other regulators to develop non-root morphologies (callus, shoots, etc.). The alkaloid levels of transformed and non-transformed tissues have also been compared. An *Artemia salina* bioassay was used to test toxicity levels of different clones and normal tissues in a preliminary screening for bioactive compounds.

P-1094

Manipulation of the Maize Meristem for Transformation. M. ROSS, L. Church, V. Phillips, P. Troy and W. Gordon-Kamm. Pioneer Hi-Bred International, Inc. Johnston, IA 50131

As a means of alleviating the severe genotype limitations that hamper maize callus-based transformation, recent work has focused on developing a transformation method for use with maize inbreds. Microprojectile bombardment into the vegetative shoot meristem of immature maize embryos followed by direct germination yielded high frequencies of mericlinal chimeras in recovered plants. Predominantly, these sectors were small and often only occurred in the first and second leaves of the plants. Transgenic plants were also obtained in which sectors continued into the tassel and ear, although the transgenes were not transmitted to progeny. Research done to overcome this problem has focused on forcing reorganization of the meristem prior to and following transgene introduction. Methods to reorganize have utilized a pre-treatment prior to bombardment, mechanical disruption of the apical dome, and nonlethal selection pressure.

P-1095

Germline transformation of maize using shoot mutiplication to enlarge chimeric sectors. K. LOWE, G. Hoerster, M. Ross and W. Gordon-Kamm. Pioneer Hi-Bred Internation Inc. Johnston, IA 50131

Direct DNA delivery into the developing meristem of coleoptilar-stage embryos produced chimeric plants at high frequency. Unfortunately, even plants with large sectors extending through the ear node and tassel failed to result in germline transmission, presumably because they were mericlinal. A shoot multiplication step stabilized and enlarged these sectors increasing the probability of a transgenic sector contributing to the germline. We used used the particle gun to deliver genes conferring either kanamycin or streptomycin resistance and GUS into the meristem of coleoptilar-stage embryos Embryos were germinated and the regions that contained the meristems were excised and cultured on shoot multiplication medium with antibiotic. Transgenic sectors were identified by their green phenotype. Transgenic plants and progeny expressing foreign genes were obtained. Transformation was confirmed in progeny using Southern analysis, NPTII ELISA, and GUS histochemical and Fluormetric assays. This method was successful using both hybrid and elite germplasm.

P-1096 Characterization of Transgene Insertion and Expression in a Glufosinate-Resistant Maize Line. T. MICHAEL SPENCER, Laurie C. Wilson, Richard J. Daines, Paul Julstrom, Rita Mumm, and Christopher E. Flick. DEKALB Genetics Corporation, Discovery Research, 62 Maritime Drive, Mystic, CT 06355

Detailed molecular and biochemical analyses were performed on a glufosinate-resistant maize line. The transgenic line, designated B16, was generated by microprojectile bombardment of cultured maize cells with a plasmid containing the *bar* gene under control of the 35S promoter. The B16 transformation event was shown to consist of a single insertion on the long arm of chromosome three. The insertion contained a single copy of *bar* and the plasmid DNA had undergone rearrangement and/or recombination, presumably prior to integration. Analysis of *bar* expression levels in B16 plants was performed using a polyclonal anti-phosphinothricin acetyltransferase (PAT) antibody. Quantification of PAT from Western blots indicated that PAT levels in leaf, stalk, and root were approximately 0.1% of total protein. PAT levels were over ten-fold lower in B16 seed; PAT was not detected in B16 pollen. Field analysis of several B16-converted hybrids showed that B16 plants were completely resistant to the recommended field application rate of glufosinate.

P-1097

CIMMYT EFFORTS TOWARDS THE PRODUCTION OF TRANSGENIC TROPICAL MAIZE WITH ENHANCED INSECT RESISTANCE

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CIMMYT, Apdo. Postal 6-641, 06600 Mexico, D.F., MEXICO

A project entitled "Enhanced insect resistance based on Bt toxins in maize for environmentally safe production in developing countries" was initiated by the International Maize and Wheat Improvement Center (CIMMYT) in May 1992. The main objectives are: (1) to use advanced biotechnology to generate tropical maize germplasm that posses enhanced and effective resistance to major insect pests of the crop and (2) to provide the improved germplasm to breeders and farmers in developing countries.

The principal activities are: (1) to develop protocols for screening toxins from *Bacillus thuringiensis* (Bt) strains against tropical borers species; (2) to define the culture conditions necessary for regenerating tropical and sub-tropical maize inbreds relevant to the CIMMYT Maize Program; (3) to transform selected genotypes with Bt gene constructs and (4) to evaluate the resistance of the transformed plants to insects.

Partially purified spore/crystal complexes and purified toxins from cloned *cry* genes from Bt strains have been screened for toxicity against four corn borer species: *Diatreae grandiosella, Diatreae saccharalis, Spodoptera frugiperda* and *Heliothis zea*. The genes for toxins found to be extremely toxic to each of the four insects will be isolated, sequenced and made available in proper constructs for transformation and testing in transgenic maize varieties.

Highly regenerable, embryogenic calli were obtained in 50% of tropical and sub-tropical, 75% of the highland, and 87% of the midaltitude inbreds evaluated. Type II callus was developed from two selected CIMMYT inbreds CML67 and CML216. Using the optimal microprojectile bombardment system these inbreds were transformed with a syntethic gene encoding an insecticidal crystal protein from *Bacillus thuringiensis* and a selectable marker gene for antibiotic resistance. Maize TO progeny were generated and will be analysed soon.

P-1098

HMGCoA Reductase Gene: A unique RFLP marker for the varietal identification of Oryza sativa.L.

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Rice(Oryza sativa L) is one of the most important crop plant all over the world.Rice,though,principally a tropical crop requiring high temp & humidity for its growth but in spite of its large productivity, most of the continent are deficient in rice as beacuse a large portions of the cultivated plots may be turned into saline or deep water tracts due to human & environmental interferences.
So,to improve both the quality & quantity of rice, development of a good marker is most important. Here we are using HMGCoA reductase gene probe as a DNA molecular marker for varietal identification. Mainly the different indica rice cultivars(IR36, SR26B etc.,) are selected along with some local cultivars(Bhasamanik,Matla etc.,).At the same time salt and submergence tolerence characters are also taken into consideration.
After isolation & purification of DNA from different rice varieties they were digested with different restriction enzymes and then blotted on specific membrane filter bed & when hybridised with the required probe then they showed the specific RFLP pattern.
We greatly acknowledge the financial assistance of The Rockefeller Foundation,CSIR-INDIA and UGC-INDIA.

P-1099

TISSUE CULTURE STUDIES ON THE NODAL EXPLANTS OF PSIDIUM GUAJAVA (GUAVA)

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ABSTRACT: A Tissue-Culture technique for rapid vegetative propagation has been developed. Nodal Sections from the sprouts of adult field grown trees and young glass house grown plants of guava have been used as explants. Nodal sections were inoculated on MS medium supplemented with various concentrations of BAP in the range of 1-5 mg^{-1}. Nodal sections from young plants showed multiple shoot formation with 1 mg^{-1} BAP alone after two weeks of incubation, whereas Nodal-sections from adult trees showed micropropagation with 4 mgl^{-1} BAP after 3 weeks aof incubation. Use of 0.5 x PVP i.e., Polyvinyl poly pyrolidone enhanced the efficiency of micropropagation from nodal explants.

P-1100

Micropropagation of *Milicia excelsa*.

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We developed a micropropagation protocol for *Milicia excelsa* Welw., a major tropical forest species in Ghana. Axillary buds of *M. excelsa* were cultured on a shoot elongation basal medium (SEBM) [Woody Plant Medium (WPM), half-strength MS vitamins, 2% sucrose, 0.7% Difco Bacto-agar] supplemented with 2.2 μM N^6-benzyladenine (BA) + 0.27 μM naphthalene-acetic acid (NAA) for shoot elongation. Shoots stumps were then transplanted to the SEBM supplemented with 2.2 μM BA and 0, 0.054, or 0.27 μM NAA to determine a suitable growth regulator treatment for shoot multiplication. The multiplied shoots were harvested and shoot stumps were transferred to a fresh medium every four weeks for four months. The harvested shoots were placed on rooting media where sucrose levels (0.2%, 0.3%), types of growth regulators (NAA, IBA), and concentrations of growth regulators (5.4 μM and 10.8 μM of NAA, 4.9 μM and 9.8 μM of IBA) were combined factorially. Percentage rooting, root dry weight, root length and numbers were evaluated based upon each combination of the factors. IBA treatment resulted in 38% more root length on average than NAA treatment. The highest rooting percentage (93%) of micro-shoots was obtained on WPM supplemented with full-strength MS vitamins, 0.7% agar, 2% sucrose, and 9.8 μM IBA. All rooted micro-shoots survived after transplanting to a soil medium.

P-1101

Effect of Inoculation with Four Strains of the Beneficial Bacteria *Azospirillum* Spp. on Proteins and Peroxidase Content in Cowpea (*Vigna unguiculata*) Calli. L. Alcaraz-Meléndez, S. Real-Cosío and Y. Bashan. Department of Microbiology, Division of Experimental Biology, The Center for Biological Research (CIB), P.O. Box 128, La Paz, B.C.S., 23000, Mexico.

The inoculation of Plant Growth-Promoting Rhizobacteria of the genus *Azospirillum* changed some plant morphological parameters in intact plants. Changes in plant metabolism have been scarcely documented, and it is has not been established whether this bacteria creates harmless relationships with its host plant.

Cowpea calli were grown in optimal growth conditions in Murashige and Skoog medium with 3% sucrose sterilized by filtration, and inoculated with 4 *Azospirillum* strains belonging to 3 bacterial species (*A. brasilense* Cd, *A. brasilense* 245, *A. lipoferum* JA4 and *A. halopraeferens* AU10) . Ten days later, we detected the following physiological changes: (i) Inoculation did not increase the protein content of any of the inoculated calli, but sometimes decreased it. These changes were within the range of 10% of non-inoculated calli. (ii) Although changes in peroxidase activity were strain-related and sometimes statistically significant, they were within the range of ± 8%. (iii) All strains significantly increased phenol production in the calli from 50 to 120% . We believe this data provides evidence that *Azospirillum* species are non-destructive to cowpea calli, but may accelerate the physiological aging process.

P-1102

Improved Embryogenesis and Plants Regeneration from Isolated Microspore Culture of Wheat (*Triticum aestivum* L.). T. HU and K.J. KASHA. Dept of Crop Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

The objective of this paper was to develop a simple and efficient procedure of isolated microspore culture of wheat for breeding and gene transformation use. Pretreatment of anthers in FHG macronutrients plus 0.4M mannitol for the periods of 0, 4, 7 and 10 days showed that a 7 day pretreatment significantly increased the numbers of regenerated green plants. For microspore isolation after pretreatment, four mechanical methods were tried: Vortexing, Sonication, Magnetic plate stirrer and Maceration. Isolation by Vortexing resulted in increased numbers of dividing microspores. As auxin source replacing 1 mg/L IAA, 0 to 10 mg/L PAA in induction media indicated that the best response in microspore culture was 4.0 mg/L. Isolated microspores were co-cultured with 20 wheat ovaries on the top of semi-solid modified MS induction media for 30 days. This system dramatically increased the number and quality of embryos. The frequencies of viable microspores that underwent division and formed embryos were as high as 94% and 2.4%, respectively. Over 2000 embryos and 200 green plants were achieved per plate (100 anthers of cv. Chris). Root tip chromosome number counts and fertility checks of 114 green plants showed that 82% were spontaneously doubled haploids and 75% were completely fertile. These results improve the potential for the use of isolated microspores in genetic transformation and breeding programs in wheat.

P-1103

Protoplast Isolation and Fusion in Two Seedless Orange Cultivars

Polyembryony, extreme heterozygosity, sterility and seedless capacity often cause serious problems in Citrus breeding. Protoplasts of two seedless cultivars, Washington Navel and Valencia oranges, have been fused. Nucellar callus was initiated from in vitro cultured germinated ovules from 24-week-old fruits of Washington Navel on MTE, MTB and MTG media. Nucellar Valencia orange plantlets were obtained from undeveloped ovules of 20-week-old fruits germinated on MS+500mg/L ME medium, and transferred onto MT basal medium +1mg/L GA_3 and MT basal medium +40mg/L ADE+1mg/L GA_3 +500mg/L ME. Protoplasts of Washington Navel were isolated from white friable embryogenic callus, and protoplasts of Valencia from nucellar leaves.

In their isolation, the effects of 13 enzyme types and mixtures were examined. High yields of protoplasts were obtained using CPW with 13% mannitol and containing Cellulysin and Macerozyme. High viability of isolated protoplasts was shown using fluorescein diacetate solution under the UV light microscope. Protoplasts of the two cultivars at densities of 10^5 per ml were mixed and fused with the aid of sterile PEG. After 15 min, many fused protoplasts were clearly visible.

P-1104

Unexpected Morphogenetic Responses Induced by Auxins Alone in Mammillaria san-angelensis, a Severely Endangered Cacti. T. MARIN and A. Rubluo. Institute of Biology, UNAM, México 04510.

The morphogenetically inhibitory action of auxins in Cactaceae has been suggested by some authors. The aim of this work is to assess the effect of auxins in long-term in vitro subcultured M. san-angelensis tissue. Sections of plantlets which were obtained from long-term in vitro subcultures (up to 5 years) were used as explants. The explants were inoculated into MS supplemented with several auxins (IAA; 2,4-D; IBA; NAA and Picloram) at (0,2,4 and 6 mg. l^{-1}) and incubated at 27 °C, 16 hours of ligth (3.2w. m^{-2}) and 8 hours of darknees. Morphogenetic responses were monitored after two months of culture. Contrary to the expected, callus proliferation did not occur in all the explants exposed to auxins. Instead shoot formation was apparent in various treatments, and some of them were able to develope buds and shoots without any callus. An inverse correlation between shoot formation and callus presence was observed. Best performance was recorded in presence of IAA (6 mg.l^{-1}) in which an average of 8.5 shoots per explant was registered. Root formation was recored only in low frequency and only in presence of IAA a IBA. The probable mechanisms involved in this unexpected auxin action are discussed.

P-1105

Embryogenesis from Callus Culture of Immature Zygotic Embryo of *Commiphora wightii*.

Anita Kumari. Laboratory of Bio-Molecular Technology, MLS University, Udaipur, Rajasthan 313 001 (India).

Commiphora wightii (Arnott) Bhandari (Burseraceae) is a prominent medicinal plant of India. The study reports indirect somatic embryogenesis from the callus of immature zygotic embryo of *C. wightii*. The initial callus was obtained on B_5 medium supplemented with 2 µM 2,4,5-T and 2.3 µM kinetin. Two passages of 4 weeks each were allowed for aging of the callus. This callus when transferred on the same medium with reduced 2,4,5-T (1 µM) produced embryogenic callus. The B_5 medium added with either one of the three combinations — (i) IBA (0.49 µM) + Kinetin (2.3 µM); (ii) Kinetin (8 µM); and (iii) BAP (8 µM) — produced asynchronous growth of somatic embryos from the embryogenic callus. The embryos transferred on B_5 medium with reduced 0.0292 M sucrose and charcoal (1 gl^{-1}) or manitol (0.165 M) matured to cotyledonary stage. The cotyledonary embryos germinated readily on PGR-free B_5 medium. The effects of different concentrations of auxins and cytokinins on embryogenesis were studied. The effects of stress-agents, charcoal and manitol, were also analyzed.

P-1106

Direct Somatic Embryogenesis from Immature Zygotic Embryos of *Commiphora wightii*.

Anita Kumari. Laboratory of Bio-Molecular Technology, MLS University, Udaipur Rajasthan 313 001 (India)

Commiphora wightii (Arnott) Bhandari (Burseraceae) is an endangered medicinal plant endemic to the Thar desert. It is well-established that the plant product oleo-gum-resin possesses antiarthritic, anti-inflammatory and hypo-cholesteromic properties. The present study attempted to induce embryogenesis in *C. wightii* and to select a suitable medium and combination in plant growth regulators (auxin and cytokinin) to induce embryogenesis and germination. The direct somatic embryogenesis *in vitro* was induced from immature zygotic embryos of *C. wightii* using B_5 basal medium with low concentrations of IBA and kinetin. The effect of factorial concentrations of IBA x kinetin in B_5 medium on somatic embryogenesis was studied. The maximum number of embryos were recorded on B_5 medium with 0.245 µM IBA and 0.46 µM kinetin, which was also found suitable mediium for cyclic somatic embryogenesis. The presence of kinetin — even though not essentiala for embryogenesis — had a significant stimulatory effect at low levels (0.23 and 0.46 µM). The high concentration of kinetin (1.15 µM) was inhibitory suggesting a quadratic relationship. IBA seemed to have triggered maximum embryogenesis at 0.245 µM level and all higher concentrations had a linear declining effect. The somatic embryos germinated when transferred on PGR-free B_5 medium. However, a significantly higher level of proportion of germination was achieved on B_5 medium with 1.15 µM kinetin and 0.49 µM IBA.

P-1107

Regeneration Ability via Somatic Embryogenesis in Pigeon pea [Cajanus cajan [L] Millsp]. PARAMPREET KAUR and J.K.Bhalla, Cytogenetics and Tissue Culture Laboratory, Department of Botany, Osmania University, Hyderabad -500 007, INDIA.

A study was made on the cotyledon explants of two cultivars of Pigeon pea viz Hy3C and ICPL 89021 to test their regeneration ability. For the experiments seeds were sterilised and soaked for 20 hours, and the distal halves of the cotyledons were cultured. Different media and concentrations of hormone adjuvants such as 2,4-D+kn; NAA; NAA+BAP; NAA+BAP+kn; BAP; IBA and GA3 were tested. Among the media tested [MMS] was found to be the most suitable. This medium comprised of MS salts and vitamins of B5. The auxin NAA at low level [1mgl-1] along with high levels of BAP [5mg 1-1] and kn [0.5mgl-1] had a promoting influence on the induction of somatic embryos. Reduced levels of hormones favoured maturation of somatic embryos. These embryos germinated on the germination medium comprising of GA3 [0.75mgl-1] and IBA [0.5mg 1-1] Complete plants have been obtained both by direct somatic embryogenesis from the excised segments of cotyledons as well as indirect somatic embryogenesis by differentiation of callus cultures of the same explants. The results obtained with both the cultivars were similar thereby suggesting the genotype independent nature of the explant response.

P-1108

Factors affecting the formation of embryogenic callus and embryogenesis from undeveloped ovules of Valencia and Washington Navel orange

Undeveloped ovules of immature and mature fruits of Valencia and Washington Navel orange were cultured on six media. MS medium+malt extract increased the germination of ovules. Germinated ovules were transferred to a range of media, two of which, based on MT medium+GA3, were effective in stimulating the development of embryos from both cultivars to produce a large number of plantlets. A small amount of callus was also obtained from the germinated ovules, embryos, pseudobulbils, and the hypocotyl region of embryos. 4-week cycles of callus subculture caused the proliferation of this callus on a modified MT basal medium supplemented with 500 mg/L malt extract+1550mg/L glutamine to produce friable white callus, especially from Washington Navel orange. The callus proved to be embryogenic, forming embryos at the heart and torpedo stages and eventually producing multiple shoots.

P-1109

Genetic Manipulation of Certain Grain Legumes Using Tissue culture Methods.D.RAO and*Ayodhya Ramulu.Ch.(DR,ARC*). Department of Botany, Kakatiya University, Warnagal-9.*present adds. 240,PABL. 1201.West Gre.Driv.Urbana.IL.U.S.A.

Grain legume crops are important source of high quality of protein in human diet and also important component of sustainable agriculture in rainfed areas. Increased awareness of the nutritional value of legumes has brought modern agriculturist to reconsider their strategies for genetic manipulation of these crops.Crop modification has undergone revolution in the past few years due to development of new in vitro culture systems. Seed material of grain legumes i.e Vigna radiata (L.)Wilczek,Vigna sinensis (L.) Savi and Cicer arietinum(L.) obtained from NSC (Wgl.)and disorganised callus cultures were induced using the one weak old aseptically dark grown seedling explants. The callusing media consisted of B5(Gomborg etal.,1968) based nutrient medium supplemented with 2.5mg/L Benzyl aminopurine, 1.0mg/L Napthalene acetic acid and 1.0 mg/L Kinetin. And the friable callus was exposed to certain environmental mutagenic compounds like Anthio and Endosulfan with 20,40,and 60 ppm concentrations. The hypocotyl derived greenish friable callus was subculture to selective and Non-selective medium at 4 weaks intervals till no inhibition of growth was observed. Anthio at 60 ppm and Endosulfan at 40,60 ppm has significantly reduced the growth of callus to 45% and 60% respectively. At 600ppm of Endosulfan tolerance in Vigna the growth was almost ceased and callus was turned dark brown in three weaks of growth period of incubation.

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P-1110

Utilisation of Thidiazuron (TDZ) for Development of Effective Regeneration System in *Lycopersicon esculentum* Mill.

T. ZAKIR, E. Iankova, L. Sthereva, N. Zagorska.
Institute of Genetics, Sofia 1113, Bulgaria

Different concentrations of kinetin, BAP, zeatin, TDZ in combination with ANA and IAA were tested to develop an effective regeneration system in tomato which is necessary in the investigations on genetic transformation and gene manipulations. Experiments with seven tomato genotypes were carried out. Stem and leaf explants of 30-day old plants grown in vitro and hypocotil and cotyledon leaves of 7-day old plants were used. Callus was induced on all of the tested nutrient media. However, organ induction depended on the genotype, the kind of explant as well on the nutrient medium. Cotyledon leaf explants of the hybrid Roma x Bella showed the best results. TDZ was most effective compared to the other hormones. After carrying out a number of experiments at one and the same conditions, TDZ showed 10 times higher ability to induce regeneration than BAP in the hybrid Roma x Bella and 7 times higher in the hybrid Roma x UC-82A. The best results were observed in concentration 2mg/l TDZ combined with 0.5mg/l IAA from the tested four TDZ concentrations. The influence of important factors such as ligh duration, type of explant and its orientation upon the nutrient medium on the organogenetic potential was established. Plant regeneration conditions were optimised. Effective regeneration system which will be used in tomato gene transformation experiments was developed basing on the results obtained.

P-1111

Biological control of crown gall on peach. M. Pérez-Zavala, J.M. Haro-Hernández, M. Ramos-Parra, O. Vázquez-Martínez and L. Valera-Montero. Instituto Tecnológico Agropecuario 20 Ap. Post. 1439 Aguascalientes, Ags., México.

Crown gall is present in many peach orchards of Aguascalientes, and so far, there is no available control for this problem. Therefore, this work was oriented to test the biological control of Agrobacterium tumefaciens infecting peach (Prunus persica L. Batsch) by using its natural enemy Agrobacterium radiobacter strain K84. To accomplish this, the local strain of A. tumefaciens was isolated and analysed for the opine group and the Stonier's test. Since that strain was found to be very similar to strain C58, we performed a series of experiments using the local strain and C58 of A. tumefaciens against A. radiobacter K84 on 400 one-year old peach trees and young Kalanchoë tubiflora. Bacterial concentrations were from 1x 10^6 to 9x10^8 bact./ml, and the combinations were 1:10, 1:3, 1:1, 3:1 and 10:1 of A. tumefaciens / A. radiobacter. These combinations were added to fresh wounds made on stem, crown and root. Special treatments included ground tumors from the commercial orchards mixed with the pot substrates in which plants with wounded roots were placed. Control plants were wounded with sterile scalpel. From these experiments we concluded that, under our conditions, A. radiobacter cannot exert a real control on the local strain and C58 of the pathogen since all of the treatments exposed to the presence of A. tumefaciens showed crown gall formation. Nevertheless, the higher percentages of tumor formation (up to 60 %) were found on those plants infected with high amounts of the pathogen (A. tumefaciens/A. radiobacter > 1).

P-1112

In vitro clonal propagation of guava tree (Psidium guajava L.). O. VAZQUEZ-MARTINEZ and E. Pérez-Molphe-Balch. Universidad Autonoma de Aguascalientes. Av. Universidad 2100. Aguascalientes, México. 20100.

Guava is the most important fruit produced in Aguascalientes that recently faces marketing problems due to diseases and high costs of production. One of the reasons hindering resolution to these problems is lack of efficient systems for asexual propagation of local guava cultivars. Therefore, the present work was oriented to obtain a reliable method for clonal micropropagation of guava. Initially axenic seedlings were the source of explants such as shoot apex and nodes, which were exposed to several combinations of BA, TDZ, IAA and GA_3 present in three different media: MS, McCown's and White's. The highest rate of shoot bud production was induced by MS with BA 2 mg/l and IAA 0.25 mg/1. Working with hypocotyls, we found low rates of adventitious shoots formation. The best responses were induced by MS with low concentrations of BA or TDZ in combination with NAA. Shoots produced with these methods were rooted on either MS plus low levels of IBA or MS free of growth regulators. Some other experiments were performed using nodal explants obtained from elite adult trees. In these experiments we found problems concerned with contamination and oxidation. For desinfection, several treatments were tried and the best way to circumvent these problems was: after several rinses, the explants were exposed to NaOCl 2.5% during 3 min, and ethanol 70% during 30 sec. Later, the explants were placed on MS containing Cefotaxime 300 mg/l and Nystatin 50 mg/l. Oxidation was controlled with ascorbic and citric acid on the inoculation medium. We found that 60% of the nodal explants were established and all of these produced 2-4 shoots each, on a medium containing BA 1 mg/l and GA_3 0.5 mg/l.

P-1113

Effects of Preparation and Storage on Gelled Medium Water Status and *in vitro* Growth of Cranberry (*Vaccinium macrocarpon*) and Grape (*Vitis vinifera* L.) Cultures. J.U. TOLEDO, M.A.L. Smith, L. Art Spomer, and D. Madhavi. Dept. Horticulture. University of Illinois. Urbana, IL. 61801.

The influence of gelled medium preparation, storage, concentration, and type on medium water status and in vitro growth of cranberry shoots *(cv. Franklin, Ben Lear, Howes, Stevens, Early Black)* and grape callus cultures *(var. Merlot)* was studied. Media gel concentration *(5, 6, 7, 8 g·L^{-1})*, storage temperature *(5, 20°C)*, storage time *(1, 30 days)*, and gelling agent *(Gelrite, Sigma agar, Bacto agar)* effects on shoot length and callus diameter were monitored weekly. Callus fresh and dry mass were determined at the end of 4 wk. Gelled media concentration increased *(due to water loss)* from 7.0 to 7.2 to 7.4 g·L^{-1} during preparation, autoclaving and cooling respectively. The concentration continued to change throughout the 30 day storage period in the lab from the original 7.0 g·L^{-1} at preparation to almost 10.0 g·L^{-1} at the end. In cool storage the concentration increased less—to 8.8 g·L^{-1} after 30 days. The stored media also yielded substandard culture performance. Experimental gel concentration did not significantly affect shoot growth or callus dry mass and diameter; however, callus fresh mass *was* significantly affected. Medium brand did not significantly influence media water status or culture performance, but substitution of Gelrite increased water availability slightly and increased shoot growth.

P-1114

Asymmetric Intergeneric Somatic Hybrid Plants Between *Lycopersicon esculentum* x *L. pennellii* (+) *Solanum melongena*. V.M. SAMOYLOV and K.C. Sink. Department of Horticulture, Michigan State University, East Lansing, MI 48824.

Intergeneric asymmetric somatic hybrids were obtained by PEG/DMSO fusion of mesophyll protoplasts of a kanamycin resistant (KmR^+) interspecific tomato hybrid of *esculentum x L. pennellii* (EP) with protoplasts of *S. melongena*, eggplant. In order to obtain asymmetric hybrid plants, elimination of tomato chromosomes was directed by application of 100, 250, 500, 750 and 1000 Gy of γ-rays to the EP donor protoplasts. Selection of hybrid calli was based on kanamycin resistance transferred from EP. KmR^+ calli were recovered at all irradiation doses used in fusions. The hybrid nature of selected calli was confirmed by PCR amplification of the NptII gene, RAPD's and Southern hybridization. Flow cytometry analysis of hybrid calli revealed that all lines that did not regenerate shoots were 6-8n polyploids. Calli ploidy level did not correlate with the irradiation dose applied to the donor. Asymmetric somatic hybrid plants were regenerated only from calli with the ploidy level close to 4n and such calli only occurred when the donor was irradiated with 100 Gy.

P-1115

A protoplast-to-plant protocol for mulberry Trees. Y.SAHOO, S.K.Pattnaik, P.K.Chand, Plant Tissue & Cell Culture Facility, Post-Graduate Dept. of Botany, Utkal University, Bhubaneswar-751004, Orissa, India.

Callus cultures were induced from hypocotyl segments of two elite indigenous varieties of mulberry, Morus indica L. var S-34 and K-2 on MS medium with 2 mg/l 2,4-D. Further proliferation of callus required a combination of 2 mg/l NAA and 0.5 mg/l BAP alongwith a supplement of 15% coconut milk and 100 mg/l casein acid hydrolysate. Cell suspension cultures were raised from friable callus portions in liquid medium and cells harvested in the exponential growth phase were used as a source of protoplasts. Incubation for 16h in an enzyme mixture of 2% Rhozyme, 1% Cellulase R10 and 0.03% Macerozyme R10 in CPW13M yielded 3.2-3.4x10^6 protoplasts/g. fwt. KM medium with suitable modifications ensured sustained division of protoplasts. Colony formation was markedly stimulated by agarose or alginate embedding of protoplasts. Callus formation occurred two weeks after colonies were placed on semisolid (0.6% agar) MS medium with 2 mg/l NAA + 0.5 mg/l BAP. Calli turned nodular and organogenic two weeks following transfer to MS medium with 0.25 mg/l BAP alone and a week thereafter green shoots developed on the callus surface. Rhizogenesis was elicited from all shoots on MS + 1 mg/l IBA within two weeks of culture. Protoclonal regenerants were hardened off on artificial soil and eventually transplanted in the open field.

P-1116

In vitro culture of tomato hybrids (Lycopersicon esculentum Mill.)

T.C.NARAYANASWAMY and N.M.Ramaswamy Department of Biotechnology, Tamil Nadu Agricultural University, Coimbatore-641 003.

Shoot tip sections of three commercial hybrids (Sonali, Rashmi and Rupali) and one cultivar of tomato (cv.PKM-1) were studied for their morphogenic responses under defined in vitro conditions with a view to find out the possibilities of clonal propagation. MS medium supplemented with BAP (2 mg/l) and sucrose (3%) was used. Shoot tip explants of all the hybrids and cultivar have initiated callus from the basal cut ends in 10 to 12 days after inoculation. Creamy white callus growth was seen at the cut end and progressed along the explant in all hybrids and cultivar. The callus proliferation was found to be more in hybrid 'Sonali' followed by 'Rashimi' and 'Rupali'. Hybrid 'Rashmi' produced multiple shoots ranging from 3-12 and an average more than five shoots per explant, whereas only three shoots were recovered in the hybrid 'Sonali'. It is seen that, hybrid, 'Rashmi' was the most amenable genotype for in vitro multiple shoot formation with as many as 12 shoots.

P-1117

Comparison of Taxol Productions Between In Situ and In Vitro Tissues. D Ravindranath, CY Hu, G Sharma, M Sahni (Wm. Paterson Coll. Wayne, NJ 07470), and NC Vance (USDA Forest Service, Corvallis, OR 97331)

Is the *in situ* high taxol yield genotype will also lead to high yield callus in *in vitro* cultures? Four clones (#1-4) of *T. brevifolia* and one clone (#5) of *T. canadensis* were used. About one hundred shoot explants from each clone were placed in cultures. Fett-Neto et al's medium (B5 medium with 8 mg/l of 2,4-D, 0.5 mg/l GA_3 and 3% PVP) and Chee's medium (Litvay medium with 20 mg/l 2,4-D) were tested. Cultures were transferred to fresh media in ten-day intervals. *T. brevifolia* clones, in general, responded the culture conditions poorly. The omitting of PVP from the Fett-Neto medium provided the best growth responses. The resultant calli, after six months culturing, were used in HPLC analysis. Clone #2 was excluded from this analysis due to poor callus growth. Twenty largest calli from each clone were extracted with MeOH:MeCl (1:1) individually. The taxol contents in all the calli of Clone #4 were too low to be detected (< 1.29 ug/g callus). The amount of taxol of individual callus from the same clone varied widely. Taxol production of clone #3, for example, ranged from undetectable to 141 ug/g callus. The average taxol contents of clone #1, 3, 4, and 5 were: 6.48 ± 6.96, 50.53 ± 39.35, <1.29, and 17.82 ± 22.28 ug/g callus, respectively. While the *in situ* taxol production of these clones were: 91, 57, 75 and 490 ug/g tissue, respectively. This work indicated that there is no apparent correlations between *in vitro* and *in situ* taxol production. Environmental factors must played more significant roles in taxol production than the genotypes. Small amounts of 10-deacetyl baccatin III and cephalomannine were detected in Clone #1 and #5, respectively.

P-1118

Promoter Type and Cell Age Influences on Marker Gene Expression in Suspension Cultured Cells Following Particle Bombardment. S.M. DETHIER ROGERS, T.Ueda, R.J. Newton and K.M. Dias. Carlson Hall of Science, Salem-Teikyo University, Salem, WV 26426-0500. Texas Agricultural Experiment Station, Department of Forest Science, Texas A&M University, College Station, TX 77843.

Plasmids PJIT166 and Dc3, carrying the B-glucuronidase (GUS) gene, were introduced into suspension cultured cells of citrus Hamlin Sweet Orange, using a helium pressured gun and gold or tungsten particles. The cells were grown in HM medium, without growth regulators. The Dc3 plasmid contains the carrot ABA-responsive promoter Dc3. The PJIT166 plasmid contains a double CaMV 35S promoter. The promoter type and cell age both affected the frequency of GUS expression, measured 48 hours after bombardment. The 35S promoter gave a four-fold higher expression level than did the Dc3 promoter. Cells in the linear growth phase, 7 days after subculture, had three to four fold higher gene expression than did older, 18 to 25 days, slower growing cells. Gold and tungsten particles gave comparable numbers of GUS-expressing cells.

P-1119

Regeneration of Soybean from Bulgarian Genotypes D. SEKULICHKA, G. Kosturkova*
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*to whom correspondence shoud be sent Institute of Genetics, Sofia 1113, Bulgaria

The production of soybean in Bulgaria has decreased nearly 10 times for the last 10 years. One of the problems is the poor resistance of the local varieties to diseases and drough. In vitro cultures can be very useful to overcome these problems. Three varieties "Boryana", "Biser", "Merit" and two lines X-62 and M-155, created through experimental mutagenesis were screned for their ability to regenerate plants. Callusogenesis was induced in cotylledons and hypocotyl explants on modified B5 and MS media, but organogenesis was not possible. Cotyledonary nodes excised from seedling grown on 1/2 MS medium enriched with 5 mg/l BAP and cultuired on MS supplemented with 0.4 mg/l BAP and 0.1 mg/l IBA were good sourse for bud formation, which varied from 76% (line M-155) to 96% (Boryana) for the different genotypes. The mean number of buds ranged between 3.1 (Boryana) and 6.8 (Merit). 29% (X-62) to 93% (Biser) of the explants with buds regenerated plants. Rhysogenesis varied as well being lowest of Biser (14%) and highest of X-62 (70%). The results show that all the genotypes have organogenetic abilities but there is a big variation among their abilities of bud formation, plant regeneration and rhysogenesis. Biser and Merit have higher regeneration ability. but poorest root formation; X-62 has lower regeneration ability and highest rhysogenesis. Probably the processes of regeneration can be improved by changing the culture medium.

P-1120

Somatic Embryogenesis and Plant Regeneration for Efficient Gene Manipulation in Higher Plants
G.M.REDDY, Centre for Plant Molecular Biology, Osmania University, Hyderabad-500 007, India.

Somatic embryoids, with bipolar root/shoot meristem, serve as a ideal source for gene manipulation and in understanding the molecular basis of growth and differentiation. In rice immature embryos of Getu exhibited high frequency of somatic embryogenesis (SE 90%) on MS with 3% sucrose, 2.5 mg/l 2,4-D. Percentage of regeneration from SE was high in TH (92%) followed by Rasi (90%) and Getu (65%) on MS media supplemented with 2 mg/l IAA + 3 mg/l BAP + 1 mg/l KN. Embryoids excapsulated in sodium alginate germinated on MS media with 1 mg/l IAA, 2 mg/l BAP + 0.6 mg/l KN (36%).Maize glumes at uninucleate stage of microsporogenesis callused with high frequency (64.5%) on MS 2 mg/l 2,4-D. The yellowish white embryogenic calli gave optimum embryoids in suspension culture with 2-3% sucrose. In triticale, calli derived from PGTS-LS1 immature inflorescence exhibited superior embryogenesis and high frequency regeneration (35%) with 4 mg/l 2,4-D, 3% sucrose and 1% mannitol. Artificial seeds germinated with a frequency of 40% on LS medium with 2% sucrose and 1% mannitol. The efficient plant regeneration is a major problem in groundnut. Somatic embryos from immature pods of four cultivars ICG 221, ICG 1908, ICG 799 and ICG 2848 on MS 4 mg/l 2,4-Dwith 2% sucrose induced embryoids with varying frequencies (30-100%), ICG 221 gave 60% embryogenesis with 8-10 SE/explant. The encapsulated synseeds germinated with 32.7% on MS + 1% sucrose + 1 % mannitol. The somatic embryoids/synseeds developed in these crop can be efficients exploited to gene manipulation.

P-1121

In vitro tumor formation on Simondsia chinensis infected by Agrobacterium tumefaciens. **O. VAZQUEZ-MARTINEZ, J. L. Moreno-Hernández Duque and L. L. Valera-Montero. U.A.A. Ave. Universidad 2100. Aguascalientes, México. 20100.**

Jojoba is a drought-tolerant long-lived evergreen shrub of the Sonoran desert. The wax ester produced in jojoba seeds has similar characteristics to whale sperm and could be used as a good substitute. The jojoba wax has many potential uses as a lubricant, Candelilla and carnauba waxes substitute antifoam, cosmetic industry, etc, but its use is restricted, due to its high production cost. Some advances on in vitro jojoba wax production have been achieved by somatic embryogenesis. The amount of wax acumulated by somatic embryos was similar to that of in vivo produced wax. Genetic transformation may be a useful tool to broaden the limits of wax production; therefore, this work was oriented to evaluate the susceptibility of jojoba in vitro to be infected by A. tumefaciens strain C58 through the induction of tumor and nopaline assays. For the in vitro experiments, axenic leaf discs and 15 mm segments of stems from young jojoba plants were cocultivated during 3 hours with 1×10^7 ,1×10^8 ,1×10^9 bacteria/ml, using 100 explants for each concentration. Subsequently, explants were placed on MS medium without plant regulators and kept at 22-25°C and 16/18 photoperiod. After 48 hours, explants were placed on MS medium suplemented with 500 mg/l carbenicilin. After 8 weeks tumors were observed on the 70-80% of the jojoba leaf discs exposed to bacterial concentrations of 1×10^8 and 1×10^9 . Stems showed 55-60% of explants with tumor formation for bacterial concentration of 1×10^8 and 1×10^9. Finally, all tumors tested showed the presence of nopalina, detected by paper electrophoresis.

P-1122

Present status of clonal propagation of elite plants of oil palm (Elaeis guineensis Jacq.) by tissue culture of immature inflorescences in Costa Rica. N.M. GUZMAN. ASD/PIPA Palm Research Program. Costa Rica. Apartado 30-1000 San José.

Adult palms of different origins: pisiferas, duras, commercial teneras and different genotypes of selected compact palms have been cloned by the use of immature inflorescence explants. Somatic embryogenesis was induced either on nodular callus or directly from the rachilla segments. Polyembryogenic masses were obtained after multiplication of two types of embryogenic tissues: compact and friable. Large number of plantlets were derived from the embryogenic cultures after differentiation of shoots and an effective rooting treatment. In some cases, approximately 300 plants were produced from a single segment of directly or indirectly produced embryogenic tissue. Acclimatization method allows 80-90% of plantlets produced to be transferred to the nursery when they are treated like seed derived plants. Plants derived from selected ortets of tenera and compact palms are now been planted in the field to increase the production of palm plantations in Costa Rica, and plants derived from duras and pisiferas are being planted to further being used in seed production and breeding purposes. Our results have shown for the first time a direct type of somatic embryogenesis for a non destructive method of clonal propagation of oil palm.

P-1123

Preliminary Studies to Determine the Micropropagation Potential of *Pistacia chinensis*. D.E. DUNN and Janet C. Cole. Department of Horticulture and Landscape Architecture, Oklahoma State University, Stillwater, OK 74078

Chinese pistache (*Pistacia chinensis*, Bunge.) is a desirable ornamental shade tree in the nursery and landscape industries. A cultivar with reliable characteristics such as fall color and branch habit would be a welcome addition to these industries. Shoot tip culture was investigated as a method of cloning Chinese pistache. Shoot tips were grown in Driver-Kuniyuki Walnut (DKW) media amended with four concentrations of IBA, 0.01, 1.5, 2.5, and 3.5 mg·liter^{-1}, and BA concentrations of 1.0, 2.5, 3.5, and 4.5 mg·liter^{-1} in a completely randomized factorial design. The 16 treatments were evaluated at eight and ten weeks for callus, shoots, and root formation. Root formation did not occur, but callus formation was documented on explants. Upon expansion, terminal and axillary buds were excised from shoots during the first two weeks that the shoots were in culture. Excised buds displayed more callus formation and higher survival rates than shoot tips. There was no significant difference in shoot or bud survival with the various media combinations. Shoots developed from the callused base of two explants.

P-1124

Clonal propagation of four medicinal plants: Ocimum americanum L. (hoary basil), O. basilicum L. (sweet basil), O. gratissimum L. (shrubby basil) and O. sanctum L. (sacred basil) through in vitro culture of axillary vegetative buds. S.K.PATTNAIK, Y.Sahoo and P.K.Chand, Plant Tissue and Cell Culture Facility, Post-Graduate Department of Botany, Utkal University, Bhubaneswar-751004, Orissa, India.

Healthy axillary vegetative buds collected from four different species of Ocimum were cultured on Murashige and Skoog's medium (MS) with 0.25-2.0 mg/l benzyl adenine (BA) and 0.25-0.5 mg/l gibberellic acid (GA_3) either individually or in combinations. In O. americanum, the sprouting of the axillary buds and development of multiple shoots was best observed on MS with 0.25 mg/l BA + 0.5 mg/L GA_3. For O. basilicum and O. gratissimum, the optimal level of BA and GA_3 in the culture medium was recorded tobe 0.5 mg/l and 0.3 mg/l respectively. In O. sanctum, high frequency sprouting of the axillary buds and development of multiple shoot was best elicited on MS with 1.0 mg/l BA + 0.5 mg/l GA_3. The shoots formed in vitro were excised and rooted on half-strength MS medium with 1.0 mg/l IBA/NAA. IBA (1.0 mg/l) was found to be most effective for O. americanum, O. basilicum and O. gratissimum. On the other hand, in O. sanctum, rhizogenesis was best induced on a NAA (1.0 mg/l) supplement. The rooted plantlets were acclimatised on artificial soil and successfully transferred to field conditions.

P-1125

Clonal Propagation and Comparative Analysis of Free Radical Scavanging Enzymes in *In Vitro* and *In Vivo* Tissues of *Gmelina arborea* Roxb. V. Ramesh Kannan and Yogesh. T. Jasrai. Department of Botany, Faculty of Science, M.S.University of Baroda, Vadodara-390002, India.

Multiple shoot formation was induced from excised nodal sectors of *Gmelina arborea* Roxb. plants on MS medium containing 6- benzylaminopurine. About 4-6 shoots were obtained within 2-3 weeks. Moreover, 7-9 shoots were formed when *in vitro* derived 1- node explants were subcultured on MS medium supplemented with still lower concentration of BAP. Very poor root induction was observed with *in vitro* shoots of *Gmelina* on half-strength MS medium supplemented with various concentrations of Indolebutyric acid (IBA). However, successful root formation was obtained when cut ends of elongated shoots were treated with IBA for very short duration and transferred to vermiculite directly. A very significant problem in various tissue culture methods is oxidative damage. This is due to the fact that the plant tissues, especially those of woody species, contain high concentrations of secondary compounds which are susceptible to oxidation. Superoxide dismutase (SOD), Catalase (CAT), and Peroxidase (PER) enzymes are known to play main role not only in scavanging the reactive free radicals but also the regeneration of the oxidised catalyst. The activities of SOD, CAT and PER were compared in *in vitro* and *in vivo* shoots, leaves and callus from leaves of sapling and mature tree. SOD activity was very high in mature tree leaves (2.025 U/mgP) as compared to *in vitro* cultured tissues. On the other hand, CAT and PER activities were comparatively higher in *in vitro* tissues than leaves of mature trees and saplings.

P-1126

Regeneration on Ability of Herbicide Tolerant Variants, R_1 Selfed and Back crossed Progenies. N.SIMEONOVA, M. Nedkovska and N. Tineva. Institute of Genetics, Department of Cell Genetics, Sofia 1113, Bulgaria

The aim of the present study was to examine the direct regeneration ability of tobacco regenerants tolerant to high concentrations (100,300mg/l) of the herbicide Glyphosate obtained by selection *in vitro*. It was investigated, also, the regeneration potential of the plants from R_1 selfed and backcrossed progenies. Direct organogenesis was induced on MS basic medium supplemented as follows: 1/ Control variant-0.5mg/l BAP; 2/ selective medium-0.5mg/l BAP, 40mg/l Glyphosate. Very good shoot development was observed directly in stem explants from Glyphosate tolerant tobacco regenerants cultivated on selective medium supplemented with 40mg/l Glyphosate. As a result of the examination on the regeneration ability of the plants from R_1 selfed and back crossed progenies it was observed also very good direct organogenesis. We have to mention that this process on the selective media it was observed later (after 2 months from the date of the inoculation), in comparison with the control medium. Our investigations showed that the tolerant variants, R1 selfed and back crossed progenies retained their regeneration ability in the present of the selective agent in the medium.

P-1127

Selection of Tobacco Somaclonal Variants Resistant to Diseases and Herbicides. N.SIMEONOVA, M. Nedkovska and N. Zagorska. Department of Cell Genetics, Institute of Genetics, Sofia 1113, Bulgaria

Somaclonal variation is already a well proved and widely used phenomenon for introducing non-specific variation into crop species, using standardised plant regeneration methods. In the course of this study it was shown that genetic variability occurred at different frequencies in tobacco callus cultures. As a result of organogenesis in seven cultivars of tobacco (Burley 21, Vranja 96, No. 4, 3942, 3974, Rilla 544, B-12 Nevrokopski) 1300 regenerated plants were obtained. Many deviations related to the morphology, habitus, shape and size of the leaves, flowers and related reproductive organs, chromosome counts and meiosis were recovered in tobacco plants regenerated from cultures. In R_2-R_4 generations more than 100 new lines were tested. Finally, 10 stable breeding lines were selected, which in comparison with the respective parent cultivars, were tolerant to *Peronospora tabacina*, *Thielavias basicola* and *TMV*. It were obtained three tobacco lines tolerant to high concentration of the herbicide Glyphosate (300 mg/l) controlling *Orobanche ramosa*. The all mentioned above tobacco lines had higher yields and better technological qualities.

P-1128

Salt tolerance in cell lines of pistachio (Pistachio vera) A.sheibani & T.A. Villiers .
Pistachio Research Inst.
P.O.Box 77175/435 , Rafsanjan , Iran .

Experiments have been conducted on the selection of salt tolerant cell lines in an Iranian pistachio root-stock. Leafderived callus multiplied on Murashige and Skoog (1962) medium supplemented with 0.5mg/L 2,4-D , an optimum concentration determined by experiment , was sub-cultured onto the same medium plus a range of concentrations of Nacl from 0.05 to 15g/L . The lower concentrations of Nacl stimulated callus growth compared with the control cultures , whereas concentrations of Nacl 5.0g/L and above caused increasing retardation of growth . Some recovery occurred in certain cultures where sectorial growth took place , apparently from individual resistant cells , giving cultures tolerant of salinity . Experiments on the habituation of cultures to salt showed that stepwise increase in salt concentration greatly improved the ability of the callus to maintain growth at higher salinities without check . The stability of the selected tolerance is being investigated . Polyethylene glycol used at concentrations isotonic to the salt media showed that pistachio is very sensitive to osmotic stress .

P-1129

Effect of some phenolic compounds on the Senescence of intact primary leaf of Bean (Phaseolus vulgaris L.) with particular reference to the activities of certain Hydrolytic and Oxidative Enzymes. *V.S. NISHAKARA CHARY, Plant Physiology Laboratory, Department of Botany, Osmania University, Hyderabad-500007, INDIA.*

Effect of 7 phenolic compounds (p-hydroxybenzoic, protocatechuic, gentisic, m-coumaric, ferulic and t-cinnamic acids, quercetin) on the senescene of intact primary leaf of bean, Phaseolus vulgaris L. was studied. All the compounds promoted senescence by lowering the three parameters of senescence i.e., chlorophylls, nucleic acids (particularly RNA) and total proteins. The stimulation of senescence by phenolics was associated with increasing activity of hydrolyzing enzymes (RNase and protease). The activity of peroxidase, polyphenol oxidase and IAA oxidase were also enhanced while catalase activity was lowered.

P-1130

Role of Hydroxyproline in salt tolerance of Rice. V.A.Chauhan and G.Prathapasenan. Plant and Cell Physiology Lab., Department of Botany, Faculty of Science, The M.S.University of Baroda, Baroda 390002, India.

The response of salt tolerant Bhoora rata (BR) and salt susceptible (GR-11) rice varieties to hydroxyproline (HP) was examined to understand the physiological basis of salt tolerance rendered by HP. Scutellum derived calli of these varieties were cultured on Linsmaier and Skoog's medium containing LD_{50} concentration of HP to obtain HP resistant tissues. Levels of Na^+,K^+,Cl^- and activity of cellulases, amylases, invertases, IAA oxidases of the cultured tissues were estimated at the end of 0,2,4 and 6 weeks of incubation on HP. Na+, K^+ and Cl^- contents increased with growth and BR accumulated more K^+ and less Cl^- than GR 11. Activity of cellulase, amylase and invertase was stimulated in both varieties by HP, while IAA oxidase activity was checked in Bhoora rata by HP. Thus it is surmised that HP may be rendering salt tolerance to tissues by favourably affecting their activity of cellulases, invertases, amylases and by helping them to maintain high K^+ content coupled with low levels of Na^+, Cl^- and IAA oxidase.

P-1131

Induction and Repair of UV-C Induced DNA Single-Strand Breaks in Repair Deficient Strain of Chlamydomonas reinhardtii. E.D. BLAGOEVA, L.M. Stoilov and S.G. Chankova, Institute of Genetics, Bulgarian Academy of Sciences., 1113 Sofia, Bulgaria

The application of repair-deficient mutant strains is a reliable approach to analyze the relationship between the cellular radio- and photosensitivity and the induction and repair of DNA damage. Our previous investigations have shown that UVS 1 Chlamydomonas reinhardtii strain, which is completely deficient in dark repair of pyrimidine dimers (1), displays approximately the same levels of radiosensitivity, single-strand breaks(ssb) induction and repair as the corresponding wild type strain 137 C(+) to gamma-rays irradiation (2). Our study was aimed at investigating whether the dark-repair deficient strain UVS-1, incapable to remove pyrimidine dimers is able to restore the initially UV-C induced ssb. Photosensitivity of the strains was evaluated on the basis of LD_{50} after UV-C irradiation (15-W germicidal lamp) of synchronized (S-phase) cell cultures. Post-irradiation recovery under the dark conditions from 0 to 8 hours was analyzed. Alkaline agarose gel electrophoresis and scanning densitometry was applied to check the levels of induction and repair of DNA ssb. Certain levels of initially induced ssb was observed. Approximately the same repair efficiency for this lesion was found in both strains during the whole period of recovery, being more pronounced in the first 4 hours of post-irradiation incubation. It seems that the UVS-1 strain, which capacity to remove pyrimidine dimers is blocked in the dark, is repair-proficient for such a primary DNA damage as UV-C induced ssb.

References: **1**. Small G., 1987, Mut. Res., 181, 31-35.
2. Chankova S. et al., 1994, Biol. Plant., 36, X.

P-1132

Effect of 2,4-Dichlorophenoxyacetic acid and Zeatin on Somatic Embryogenesis of Flax (*Linum usitatissimum* L.). A. Cunha and M. FERNANDES-FERREIRA. Departamento de Biologia, Universidade do Minho, 4719 Braga Codex, PORTUGAL.

Somatic embryogenesis was induced from hypocotyl segments of *in vitro* grown flax seedlings cultured on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and zeatin (ZEA). Three different concentrations of these growth regulators were studied in a 3x3 complete factorial design. With the exception for the highest ratio of citokinin/auxin concentration tested (16x), the percentage of induction was high in all combinations tested (> 75%). The best results, in terms of mean number of embryos per explant, were obtained with 0.1 mg/L 2,4-D + 0.8 mg/L ZEA and 0.4 mg/L 2,4-D + 1.6 mg/L ZEA. However, only with the later, true bipolar embryos were produced as confirmed after transference to phytohormone-free MS medium. The occurrence of morphologically abnormal embryos (with one, three or more and fused cotyledons) increased both with increasing concentrations of 2,4-D and with higher citokinin/auxin concentration ratios. Foliose and cotyledon-like strutures were also observed with frequencies ranging from 8 to 100 % in the highest citokinin/auxin concentration ratio.

P-1133

Factors Effecting In Vitro Flowering
In Brassica juncea L. Czern and Coss.
K. SRINIVASA CHARY and S.A. Forook
Tissue Culture and Cytogenetics Lab.
Department of Botany, Osmania University,
Hyderabad-500 007, A.P. INDIA.

Several factors are found to be effective in influencing the in vitro flowering in Brassica juncea (Indian Mustard). 23% of the regenerated plants shown in vitro flowering on selected flowering media. The same media is subjected to different external conditions to test the frequency of flowering. The temperature at 14°C was found to be very effective in increasing the frequency of flowering. 17 hour dark period at 25°C also found to increase in vitro flowering upto 15%. When the regenerated plants exposed to the red light of wave length 660 nm, the flowering frequency has been enhanced to 20%. But stupendous results have been achieved by internal factors. Addition of GA3 (4mg/1) to the selected media at the temperature 18°C increased flowering frequency to 40%. Addition of Calcium to the media also found much effective in increasing the frequency of in vitro flowering.

P-1134

Protein Characterization During Morphogenesis in Pigeonpea (Cajanus cajan (L) Millsp). K. SREENIVASU and S. Narasimha Chary, Cytogenetics & Tissue Culture Lab, Department of Botany, Osmania University, Hyderabad - 500 007, INDIA.

The Bio-chemical knowledge of regenerable and non-regenerable cultures is much useful for the production of cells competent enough to regenerate plants *In Vitro*. The Bio-chemical studies of Pigeonpea callus cultures showed differences in the protein profile.

The embryogenic callus was obtained from the de-embryonated cotyledon explant on the modified MS medium supplemented with NAA (4.0 mg/l), BA (1.0 mg/l) GA3 (0.5 mg/l) and 1% sucrose. The non-embryogenic, compact callus was obtained on medium with BA (2.0 mg/l), IAA (1.0 mg/l), GA3 (0.2 mg/l) and 3% sucrose. The organogenic and the non-organogenic calli were obtained from leaf explant on the modified MS medium fortified with BA (2.0 mg/l), IAA(1.0 mg/l), GA3 (0.2 mg/l) and BA (6.0 mg/l) and 3% sucorse respectively.

The morphogenic callus has higher protein content (about three times) when compared to the non-morphogenic callus. The protein analysis, showed the presence of specific protein (67 Kd) in the embryogenic callus and Zygotic embryo, and it is not present in the non-embryogenic callus. The organogenic callus obtained from leaf explant has a specific protein of 54 Kd which is absent in the non-morphogenic callus. The protein profile studies will be very useful as a marker system in the *In Vitro* regeneration system of the plants.

P-1135

Ureide Catabolism in N_2 Fixing Pigeon pea.
D.SREENIVAS REDDY, Gopal Reddy and H.Polasa,
Department of Microbiology, Osmania University
Hyderabad-500 007, India.

Pigeon pea (Cajanus cajan) and alfalfa (Medicago sativa) differ in their transport of fixed nitrogen from nodules to shoots. The dominant nitrogen transport compounds in pigeon pea are ureides, while amides dominate in alfalfa. The ureides, allantoin and allantoate are metabolized either by allantoicase or allantoate amidohydrolase pathway in plant tissues and serve as primary source of N for protein synthesis. Acetohydroxymate (AHM), a specific inhibitor of urease was used as probe to determine ureide catabolism. AHM was applied to the rooting system of nodulated pigeon pea (ureide symbiosis) and alfalfa (amide symbiosis) for a period of 25 days. AHM induced chlorosis, and a reduction in plant growth, dry weight and total nitrogen content, in pigeon pea, while these parameters were unaffected in alfalfa. The inhibitory effects were over come by application of combined nitrogen. Whereas the nitrogenase (EC 1.7.99.2) activity of these two plant nodules was unaffected due to AHM, urease (EC 3.5.1.5) levels were markedly reduced, consequently urea accumulated in all parts of the pigeon pea. These results indicated that AHM blocked nitrogen assimilation into plant protein. These studies suggested that the allantoicase pathway is the likely route of ureide catabolism in N_2 fixing pigeon pea plants.

P-1136

In vitro Tuberization in Sweet Potato (Ipomoea batatas L.Lam)J.E.B.P.PINTO and S.A.Figueiredo. Laboratory of Tissue Culture, ESAL,Cx.P.37,Lavras MG, BRAZIL, 37200-000.

The importance of in vitro tuberization of sweet potato is a means of international germplasm distribution or exchange as well as for the propagation of planting material. Plantlets of I. batatas 'Brazlandia Branca'were cultured in vitro on MS-revised medium in order to assess the influence of the several chemical (NAA, BAP, ABA and CCC) and the photoperiod on the induction of microtubers. Was observed among the chemical factors that there was a positive interaction of NAA and BAP on the formation of SLT (Structures Like Tubers) in the roots. The microtubers were confirmed as being tuberous roots by the presence of sporamine, a storage protein of tuberous roots this species, in SDS-PAGE. Nevertheless, it was still necessary to develop a methodology that could make possible the production of better conformed structures.We verified that neither ABA nor CCC influenced positively the in vitro tuberization, even alone or together with NAA and BAP. The photoperiod alone did not influence in vitro tuberization of sweet potato.

P-1137 Micropropagation of Onion through in vitro Bulblet Formation.J.E.B.P.PINTO and B.M.Rodrigues. Laboratory of Tissue Culture, ESAL, Cx.P.37,LAVRAS -MG, BRAZIL, 37 200-000.

We developed a micropropagation method for onion (Allium cepa L.) by the combination of initial bulb scale segments, shoot multiplication and in vitro bulblet formation. Shoots were induced on twin scales taken from the basal plate region on a modified Murashige and Skoog (MS) - medium containing BAP. The apex must be destroyed or injured to obtain axilary buds. It was necessary to restore plant individuality before further proliferation and bulblet formation. Regenerated shoots were induced to form bulbs im MS medium containing several sucrose concentrations, different temperature and photoperiod. Subsequently the bulblets were trimmed and split in half, and secondary plantlets were regenerated in the presence of a cytokinin. Bulblets formed in vitro were transferred to soil and produced viable plants. This technique is useful for the multiplication and preservation of genotype, since plantlets regenerated in this manner should be genetically uniform.

P-1138

In Vitro Propagation of Mangosteen (*Garcinia mangostana* L.) from Shoot Cultures. R.Aliudin and M.N. NORMAH. Plant Biotechnology Laboratory, Faculty of Life Sciences, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Malaysia.

The mangosteen, *Garcinia mangostana* L. (Clusiaceae) is an important fruit tree cultivated mainly in south and south-east Asia. It is one of the tropical fruit species with promising economic value. The best known practice for the propagation of the mangosteen is by seeds which are formed apomictically. Mangosteen flowers and fruits is definite seasons and seed production is at the average rate of two seeds per fruit. The limited availability of seeds and the inability to propagate vegetatively by conventional methods necessitated alternate approaches for mass propagation. Studies on the *in vitro* propagation of *Garcinia mangostana* L. demonstrate that shoot tip explants from *in vitro* seedlings are capable of forming multiple shoots. Proliferation of shoot tip was obtained on Woody Plant Medium (WPM) containing different combinations and concentrations of auxins and cytokinins. Highest number of shoots per explant (20-25shoots) was obtained from cultures grown on medium supplemented with 10nM thidiazuron (TDZ), 5μM 6-benzylamino purine (BAP) and 1μM α-naphthalene acetic acid (NAA). Rooting was achieved by transferring the individual shoots to WPM liquid medium having 30mg/l indole-3-butyric acid (IBA) for 2-3 weeks, and consequently subcultured onto medium without any plant growth regulators. About 90-95% of the shoots rooted after six weeks in rooting medium. Plantlets were acclimatized and succesfully established in soil.

P-1139

In vitro Propagation of Acacia holocersia - Forest tree. K.KAMALAKAR REDDY, P.Bhaskar and K.Subash, Department of Botany, Kakatiya University, Warangal-506009.

Acacia holocersia is a legume moderate tree native of Australia introduced into India with aim of social forest development of eroded and grassy waste lands throughout the tropics. It is quickly growing nitrogen fixing tree, provides forage, pulp, firewood and green manure and also has economic importance as it yeilds gums & tannins. It usually suffers from weak rooting poor stems and low seed production. In view of its economic importance the present investigation has been carried out. The hypocotyl and shoottip explants of Acacia holocersia were excised from two weeks old seedlings and cultured on modified M.S medium containing 2mg/l BAP. After 6 weeks most of the explants had a cluster of 5 to 8 axillary shoot buds with prolific branching. The maximum multiplication rate (30) was observed in the first subculture the rate is decreased 15 to 10 in the second subculture. The mean length of the shoots was not significantly affected by the BAP concentration during the subsequent cultures. The cultures were transferred to a half strength MS medium containing 0.05mg/l IBA. A suitable medium for micro-propogation has been established during the present study. The regenerated plants were successfully transferred to the soil.

P-1140

In Vitro Selection for Salt Tolerance: Changes in Activity and Induction of Specific Isozymes during Salinity Stress in *Oryza sativa* Cultivars. ANITA V.RATNAM* and G.M. Reddy**. * Dept. of Medicine,VAMC/UCSF, San Francisco, CA 94121.**CPMB, Osmania University, Hyderabad-500032, India.

In vitro selection for salt tolerance is a rapid tool for screening salt tolerant plants. Callus was initiated on LS medium containing 2mg/lit. 2,4D from various salt susceptible rice cultivars (SSRC) viz.; Basmati 370, Surekha and Randhunipagalu and from salt tolerant cultivars (STRC) Pokkali, Getu and SR-26-B. The proliferative callus was subcultured onto media containing 17, 30 and 50 ds/m NaCl and healthy growing calli were maintained with frequent subcultures (every 3 weeks) for 6 passages. Healthy calli were selected and cultured on medium containing 4mg/lit. kinetin and 1mg/lit. IAA and green plants were regenerated. The tolerant calli were used to study the changes in isozyme patterns induced due to salinity stress. Peroxidase isozymes with Rf values of 0.112, 0.336 and 0.44 were induced in SSRC, which could provide an alternate route for oxidation of reduced NAD, while the STRC showed constitutive expression of the same. An esterase isozyme with Rf of 0.136 was induced in both SSRC and STRC but with higher activity in the former. Two high molecular weight MDH isozyme bands with Rf of 0.084 and 0.114 were induced in SSRC which could be involved with more rapid oxidation of L-malic acid to oxaloacetate with the reduction of NAD that is involved in the liberation of ATP. Apart of isozymes, the levels of the osmoticum balancing agents like proline and choline also increased 3-4 fold under salt stress. Thus, the adaptation of calli to high salt conditions is associated with physiological changes within the cell which serves as one of the mechanisms to overcome the adverse conditions.

P-1141

In Vitro Techniques for Selection of Anthio Tolerant cell lines in Cicer arietinum (L.).
RAMULU A.CHINNALA. 240 Plant and Animal Bio-Technology Lab, 1201. West Gre.Drive.
University of Illinois at Urban-Champaign.IL.

Genetic modification of crop plants has undergone a revolution in the past few years due to development of new in vitro culture systems. Food legumes are important sourse of high quality of protein in human diet and they are also important component of sustainable agriculture in rainfed areas. The power of in vitro selection for the production of new and useful plants has not been fully exploited.The seed material were procured from NSC, Ananthapur Branch, AP,for investigations. Unorganised static cultures were induced from one week aseptically grown seedling explants on B5 (Gomborg et al.,1968) with various concentrations and combinations of plant growth regulators, good efficient callus was induced with 1.5 mg(6.78uM) of 2,4-D and 1.75 mg/L (8.13 uM) of kinetin. The friable callus was seperated after 45d growth period and the same was subcultured to a petri plate (100 X 15 mm) containing the B5 agar media with 10,20 and 30 PPM conc of Anthio (O-o-dimethyl S-(N-methyl-N-Formoyl carbamoyl methyl dithio phosphate) for induction of specific pesticide stress on callus cell lines.After 30d incubation the growth of static cultures were totally inhibited at 30PPM and became nacrotic at this conc of tolerence. At 10 PPM of Anthio effected cultures, inhibition is less than 60 percent ant at this conc the calli were separeted and kept for further investigations like morphogenesis, growth parameters and other biochemical studies.

P-1142 Comparisons of *Artemisia annua* Root Cultures and *Nephrolepis exaltata* Whole Plant Cultures in a Newly Designed Nutrient-Mist Bioreactor with Conventional Methods.

C.S. BUER[1], M.J. Towler[1], T.C. Smith[1], P.J. Weathers[1], and D. Walcerz[2]. [1]Biology and Biotechnology Department and [2]Mechanical Engr. Dept., Worcester Polytechnic Institute, Worcester, MA 01609-2280.

Comparison of aeroponic growth with conventional plant cultures and hydroponics has shown aeroponics provides a superior environment for growth including plant tissue culture and whole plants. Incorporation of nutrients and phytohormones into the mist provides a reproducible method of plant culture allowing comparisons between different conditions. Comparisons of growth of root cultures of *Artemisia annua* and shoot cultures of *Nephrolepis exaltata* will be presented using a newly designed mist reactor, shake flasks (for roots), and agar cultures (for shoots). This improved mist reactor separates the nutrient medium via an acoustic window and a layer of cooling water. These data will be presented and compared to previous data from our lab gathered during toxicity tests of the acoustic window material.

I-1001 Insect midgut cells in culture: a typical stem cell system. M.J. LOEB[1] and R.S. Hakim[2], (1) Insect Neurobiology and Hormone Lab., USDA, Beltsville, MD 20705 (2) Dept of Anatomy, Howard U. School of Medicine, Washington, D.C. 20059.

Mixed cell cultures from midguts of pharate 4th instar larvae of *Manduca sexta* contained stem cells, long columnar cells and goblet cells, and their intermediate developmental forms. Of these, only nuclei of the stem cells took up the thymidine analog, bromo-deoxyuridine. This observation shows that only stem cells divide *in vitro* and therefore must be the progenitors of the other cell types. Cultures established from midguts of wandering stage 5th instar larvae lack true goblet cells; their short columnar cells are uniformly granular. Stem cells from pharate 4th instar larvae cultured with conditioned media from mixed cell 4th instar cultures formed typical 4th instar larval cell types. The same stem cells grown with media from wandering 5th instar midgut cultures produced typical 5th instar midgut cells. Thus, the types of differentiated cells produced by stem cell cultures depended on the message contained in the source of conditioned medium. Only approximately 1-4% of the cells in a stem cell culture appear as mature cells at any one time, yet stem cells continually divide, mature, and mature cells die. Therefore, Lepidopteran midgut cell cultures appear to be dynamic systems resembling typical vertebrate stem cell populations.

I-1002 Characteristics of Midgut-Derived Insect Cell Lines. C.L. GOODMAN and A.H. McIntosh. Biological Control of Insects Research Lab, USDA, ARS, 1503 S. Providence, Research Park, Columbia, MO 65203

Cell lines derived from midgut tissues of *Helicoverpa zea* (Hz-MG8) and *Trichoplusia ni* (Tn-MG1) were characterized based on baculovirus production and protein profiles. The ability of the cell lines to replicate several species of baculoviruses was determined, including wild-type and/or clonal isolates of HzSNPV, AcMNPV, SfaMNPV, and AgMNPV. Extracellular virus (ECV) titers ranged from 10^3 to 3.3 x 10^6 $TCID_{50}$/ml for Hz-MG8, and $<10^2$ to 6.5 x 10^7 for Tn-MG1. Occlusion body (OB) production was observed in the TN-MG1 cell line for AcMNPV and SfaMNPV, with OB production in the Hz-MG8 line only being observed in very low levels (0.5% of cells) for SfaMNPV. The ability of Hz-MG8 to generate proteins from recombinant baculoviruses and its susceptibility to alkali-released viruses was also determined. Proteins generated by each cell line were analyzed using electrophoretic (PAGE, SDS-PAGE, IEF) and enzymatic techniques. General protein profiles for Hz-MG8 indicated that this cell line produces high levels of a monomeric protein having an approx. molecular weight of 60kd with an acidic pI. Conversely, protein profiles of Tn-MG1 showed that this cell line produces varying concentrations of a variety of proteins, similar to other lepidopteran cell lines. Isoenzyme analysis indicated that both cell lines exhibit unique esterase patterns. Neither cell line was shown to synthesize digestive enzymes. Thus, our results indicate that Hz-MG8 is a unique cell line worthy of further study to determine how and why it produces: (1) ECV but not OB; (2) high levels of a 60kd protein.

I-1003 Baculovirus *Ac*MNPV Induces Apoptosis in an Insect Midgut Cell Line. S.R. PALLI, G.F. Caputo, A.J. Brownright and S.S. Sohi, Forest Pest Management Institute, Sault Ste. Marie, Ontario, Canada, P6A 5M7.

Infection of SF-21 cells with *Autographa californica* nuclear polyhedrosis virus (*Ac*MNPV) or FPMI-CF-203 (CF-203) cells with *Choristoneura fumiferana* polyhedrosis virus (*Cf*MNPV) results in successful infection as visualized by formation of occlusion bodies (OBs). However, infection of CF-203 cells with *Ac*MNPV or SF-21 cells with *Cf*MNPV is unsuccessful and no OBs are seen. Infection of Cf-203 cells with *Ac*MNPV results in premature lysis of cells beginning at 12 hr post infection (pi) and most of the cells are lysed by 48 hours pi. These cells exhibit characteristics typical of apoptosis including formation of apoptotic bodies and fragmentation of cytoplasm as well as nuclei. Agarose gel electrophoresis of DNA from the infected cells showed a characteristic DNA ladder beginning at 12 hr pi. The apoptosis induced by *Ac*MNPV in CF-203 cells can be prevented by infection of these cells with *Cf*MNPV at least 6 hr prior to addition of *Ac*MNPV. These studies show that *Ac*MNPV induces apoptosis in non-permissive CF-203 cells due to the failure of synthesis of one or more proteins that block apotposis. If these proteins are supplied to the cells by infecting the cells with *Cf*MNPV prior to adding *Ac*MNPV, apotposis can be prevented. Supported by Canadian Forest Service and National Biotechnology Strategy Fund.

I-1004 Interaction Between Cell Adhesion and Apoptosis Pathways and its Role in the Cellular Immune Response in Insects. L. L. PECH and M. R. Strand. Department of Entomology, University of Wisconsin-Madison, Madison WI 53706.

Granular cells from *Pseudoplusia includens* (Lepidoptera: Noctuidae) exhibited the morphological and biochemical properties of apoptosis when incubated in medium preconditioned by plasmatocytes. Granular cells fragmented into small membrane-bound bodies. Hoechst staining of dying cells revealed that the nuclei became condensed and staining with rhodamine 123 indicated that mitochondria remained intact. DNA from granular cells cultured in plasmatocyte-conditioned medium was degraded into discrete fragments that formed a ladder when analyzed by agarose gel electrophoresis, whereas DNA from granular cells cultured in control medium remained in a high molecular weight form. When purified populations of granular cells, plasmatocytes, and spherule cells were incubated in plasmatocyte-conditioned medium, only granular cells died by apoptosis, and only medium conditioned by purified plasmatocytes, and not granular cells or spherule cells, induced apoptosis in granular cells. Granular cell adhesion to the culture plate was prerequisite for apoptosis, and plasmatocyte adhesion was required for the production of the apoptosis-inducing activity. We propose that induction of apoptosis in granular cells is part of a negative feedback loop involved in terminating capsule growth.

I-1005 Identification of Insect Cell Lines by DAF. A. H. MCINTOSH, J. J. Grasela and R. L. Matteri. USDA/ARS/BCIRL, 1503 S. Providence, Columbia, MO 65203.

The identification of insect cell lines is of paramount importance because of the ever increasing number of cell lines being established and their usage in biology in such areas as genetics, biochemistry, physiology, classical virology and DNA recombinant technology. In the present study 20 cell lines spanning the Orders Lepidoptera, Diptera, Coleoptera and Homoptera were analyzed by DNA Amplification Fingerprinting (DAF). Cell lines were grown in T-75 cm^2 flasks in their respective media at 28°C. DNA was extracted from approximately 3-5 x 10^6 cells. Each DNA sample was analyzed in three separate PCR reactions, using distinct oligonucleotide primers. PCR products were analyzed by electrophoresis using 2.5% metaphor gels. Markers ranging from 50-1000bp were included in each run. Gels were stained with ethidium bromide and visualized under UV light. Distinctive fingerprint patterns were generated for each cell line that enabled its identification with the following exceptions (which may reflect cell line mislabeling or cross-contamination? closely-related species?). *Mamestra brassicae* and *Plutella xylostella* cell lines gave the same pattern as a *Trichoplusia ni* (TN-CL1) cell line. Also a *Spodoptera exigua* (UCR-SE-1C) clone gave the same identifying pattern as a *Spodoptera frugiperda* (SF-21) cell line. The profiles of *Aedes aegypti* and *A. albopictus* were indistinguishable. The fingerprint pattern appears to be a stable characteristic since comparison of low and high passages of a particular cell line gave the same pattern. DAF will be useful in establishing identities of cell lines carried in laboratories.

I-1006 In Vitro Studies with the Corpora Allata of *Manduca sexta*. (*)B.G. Unni., Biology Department, Texas A&M University, College Station, TX; (*)Biochemistry Department, Reg. Res. Laboratory, Jorhat 785006, Assam, India.

The juvenile hormone (JH) output of Corpora allata (ca) of V instar larvae and 3 day adults of *Manduca sexta* was measured in vitro using a radiochemical assay (Unni et al. (1991) Arch. Insect Biochem. Physiol. 17, 129). Hemolymph from pharate Vth instars in TC-199 increased JH production 4 times compared to controls. A mixture of JH acids (I, II and III) added to hemolymph of pharate V instars increased total JH production to 14.97 pmol/hr/pr ca, which was approx. 8-10 times more than controls. The total JH production elicited in the presence of JH acid (lnM) alone was only 3.06 pmol/hr/pr ca. Allatotropin analogue (20nM) increased JH production approx. 4 times in a 3-6 hr incubation, and approx. 6 times in either 6-9 or 9-2 hr incubation periods. These studies provide a better understanding of the mechanism of action of these substances at the molecular and cellular levels. Sincere thanks are due to the Director for permission to communicate this paper, and Professors K. H. Dahm, G. Bhaskaran and Tim Hayes for use of laboratory facilities at Texas A&M University.

I-1007 Inhibitory Effect of Niclex on glycogen of Tape Worm (Neokrimia singhia) M.R. SIVA SAI KUMARI and Ratnamala Rao, Department of Zoology, Osmania University, Hyderabad-500 007 (AP) INDIA

Glycogen is a main reserve polysaccharide in helminth parasites. The glycogen was estimated quantitatively in immature, mature and gravid regions of Control and treated Parasites. High content of glycogen was present in the mature region followed by gravid and immature regions. The variation in the glycogen content may be a reflection of differential rate of metabolism along the strobilae related to the regional differences in anatomy and permeability in tape worms. During the treatment of anthelmintic drugs; Niclex. There was a general depletion in the glycogen content in immature, mature and gravid regions of the parasite. It may be indicated that depletion of glycogen storage capacity which may be attributed to the diminished uptake of glucose or due to inhibition in glycogen synthetic direction.

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Take A Look At Denver

Plan on attending this exciting 1995 Congress in Denver, CO. The Program Committee is currently soliciting "Hot Topics" Abstracts for a special poster section at the Meeting, as well as publishing these abstracts in the Addendum Booklet to be distributed at the Meeting. We look forward to seeing you in Denver!

Denver is the largest city within a 600-mile radius—an area almost the size of Europe. For more than 125 years, it has been the cultural, shopping, and entertainment capital of this vast region. The city intends to continue its leadership into the next century by building a light rail system, a 50,000-seat Major League baseball stadium, a downtown theme park, and a new library to house five million volumes. Already, Denver has completed the world's most efficient airport and Tropical Discovery, the new exhibit at the Denver Zoo that encloses a complete, two-acre rain forest.

History

Denver was born during the great "Pike's Peak or Bust" gold rush of 1859 after flakes of placer gold were found at the confluence of the South Platte River and Cherry Creek. In its first few years, the city survived a flood, several major fires, Indian attacks, and even raised an army that defeated an invading force of Confederates from Texas during the Civil War. With the discovery of more gold in the mountains, Denver became a boom town. Saloons, gambling halls, and wagon trains lined the mud-filled streets, and every outlaw, gunman and desperado in the West made at least one visit to the Mile High City. The turn of the century brought respectability as the wealth of the mountains was poured into parks, fountains, statues, tree-lined boulevards, and elaborate mansions, making Denver the most elegant city within a thousand-mile radius—the "Queen City of the Plains." The most recent boom was spurred in the 1980s by energy development, during which Denver's population nearly doubled. After the oil bust of the late '80s, Denver's economy has seen six years of steady growth, at a time when the rest of the country has been less financially stable.

Location

Geographically, Denver is in the middle of the country, just 340 miles from the exact center of the continental United States. The city sits on high, flat plains 12 miles east of the Rockies. One hour west of Denver, you can drive 14,240 feet above sea level on the highest auto road in North America, but the city itself is flatter than Manhattan.

Population

Denver is one of America's fastest growing metro regions, with 500,000 people in the city limits and two million in the metro area. The median age is 32.6 and more than a third of residents are between 15 and 35, giving the city a youthful look and flavor. Denver has the second highest number of college graduates per capita (after Washington, D.C.) and the nation's most highly educated downtown workforce. All segments of the active population enjoy the nation's largest city park system.

The Mile High Experience

Denver really is exactly one mile high. You can stand 5,280 feet above sea level on the west steps of the State Capitol. Most people don't feel the altitude in Denver, but some feel it in the mountain resorts, which are 8,000 to 10,000 feet above sea level.

Metro Denver Attractions

- Denver Botanic Gardens
- Coors Brewing Company
- City Park
- Red Rocks Amphitheater
- Tiny Town
- The Denver Zoo
- The Arvada Center for the Arts and Humanities
- Buffalo Bill's Memorial Museum and Grave
- Hyland Hills Water World
- Heritage Square

Downtown Attractions

- The Denver Performing Arts Complex
- 16th Street Mall
- Colorado State Capitol
- The Children's Museum of Denver
- Museum of Western Art
- Black American West Museum & Heritage Center
- Larimer Square
- Confluence Park
- Molly Brown House

What's New

Denver International Airport is now open. For baggage-handling during the first months of operation, the airport will use a combination of its computerized system and more traditional methods. The entire computerized system, which caused at least three delays in the airport's opening over the past year, is scheduled to be operational by August.

The St. Louis-based HBE Corp. has purchased the 740-room Radisson Hotel and an adjacent building in Courthouse Square, two blocks from the Colorado Convention Center, and is combining them into a 1,200-room Adam's Mark hotel (the SIVB meeting headquarters), with a restaurant, nightclub, retail space and park area. Construction on the two-year project would begin in June 1995.

1995 Congress on In Vitro Biology

Exhibitor's List

Advanced Instruments, Inc.
Norwood, Massachusetts

Bio-Rad Laboratories
Hercules, California

BioWhittaker, Inc.
Walkersville, Maryland

BTX
San Diego, California

Carl Zeiss, Inc., Microscope Division
Thornwood, New York

Cell Tools Inc.
San Francisco, California

CellStat Technologies, Inc.
Palo Alto, California

Collaborative Biomedical Products/
Becton Dickinson Labware
Bedford, Massachusetts

Duchefa Biochemie BV
Haarlem, Holland

European Collection of Cell
Cultures
Salisbury, United Kingdom

Fisher Scientific
Pittsburgh, Pennsylvania

Forma Scientific, Inc.
Marietta, OH

Harlan Bioproducts for Science, Inc.
Indianapolis, Indiana

Intermountain Scientific Corporation
Kaysville, Utah

JRH Biosciences
Lenexa, Kansas

Kluwer Academic Publishers
Nerwell, MA

Life Technologies
Gaithersburg, Maryland

Millipore Corporation
Bedford, Massachusetts

Miltenyi Biotec, Inc.
Auburn, California

Olympus America Inc.
San Jose, California

Osmotek Ltd.
Rehovot, Israel

PAA Laboratories, Inc.
Newport Beach, California

Pharmacia Biotech, Inc.
Piscataway, New Jersey

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Sarasota, Florida

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Monrovia, California

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Southland Cyrogenics, Inc.
Carrollton, Texas

Technical Instrument Company
San Francisco, California

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